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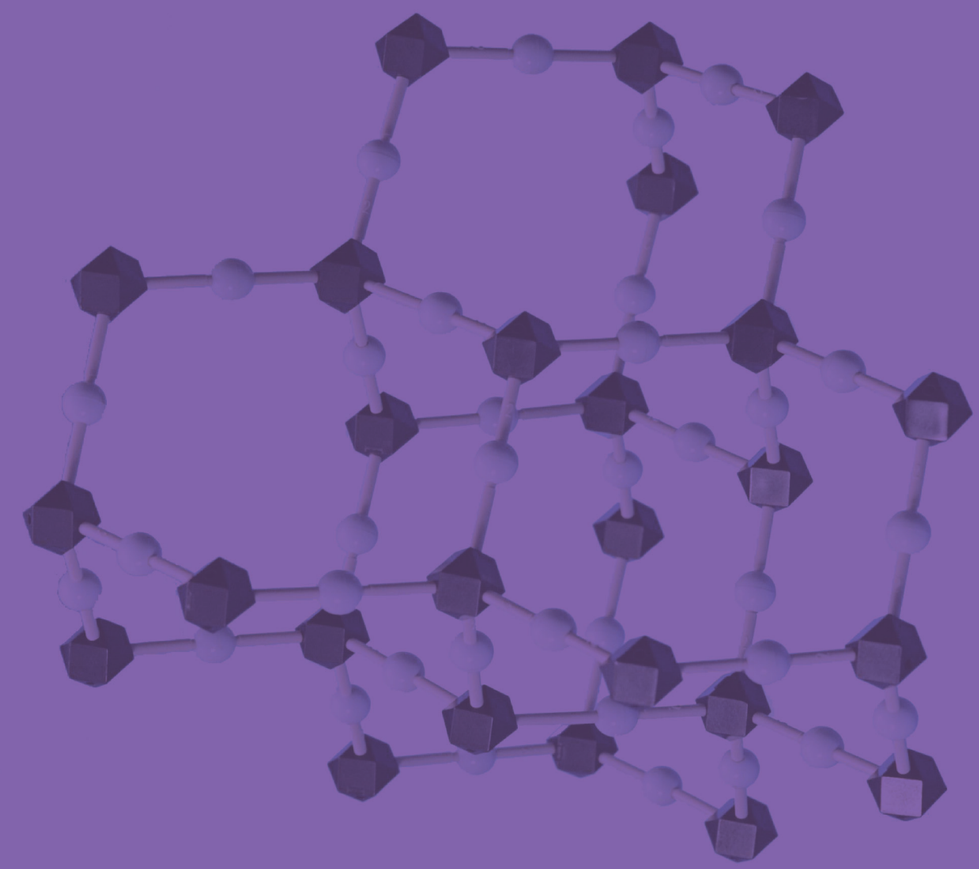

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CONTENTS

The New Concepts to Big Bang and to Black Holes: Both Had No Singularity at All (Part 1)

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Abstract: 1. Our Universe was born from Quantum Micro Black Holes (its mass ≈ 10^{-5} g), but not from Singularity or Big Bang of Singularity. 2. No Singularity existed in star-formed Schwarzchild's black holes, a steady mini black hole (its mass ≈ 10^{15} g) of long lifetime would certainly exist inside as a core to obstruct the collapse of energy-matters to become Singularity. The steady mini black hole (m_{om} ≈ 10^{15} g) in black holes instead of Singularity called by General Theory of Relativity could resist the gravitational collapse. [Nature and Science. 2004;2(4):1-10].

Key Words: universe; singularity; big bang; black holes; Plank's era: cosmology:

Part One. Our Universe was born out from quantum micro black holes (QMBHs, its mass $m_t \approx 10^{-5}$ g), but not born out from Singularity or "Big Bang" of Singularity

Introduction: In part one of this article, based on some general laws of astronomy and physics, the calculated results could prove that our present expanding Universe was impossible to be born from Singularity or from the Big Bang of Singularity but from the Big Crunch of pre-universe.

Once pre-universe collapsed to (t = -10^{-43} s, T = 10^{32} k) of Plank's Era (see figure 1 on next page), every particle and radiation simultaneously broke off its gravitational linkage between its closest neighbors and stopped their collapse at the state of no gravity. Furthermore, every particle at that moment would exactly become a quantum micro black hole (QMBH, its mass $m_t \approx 10^{-5}$ g), their presences jointly obstructed the pre-universe from collapsing into Singularity and directly led the disappearance of pre-universe in the quantum field of Plank's Era. The lifetime of 10^{-43} s of every QMBH was just synchronous with the disappearance of pre-universe. After that, the total small bangs caused by all new QMBHs born from Plank's quantum field would make up an "inflation" (or so-called Big Bang) and formed the present expanding universe. It was the genesis of our present Universe. The process above showed the changed process from the disappearance of old universe to the genesis of new universe. Other conclusions of part one can be seen in conclusions of 7th paragraph. (< > number of reference).

1. The Laws and formulas of Our Universal Evolution

The laws of our universe's evolution can be simply and precisely described by two different methods, which are based on the achievements of modern physics (GTR & particle physics)and astro-cosmology.

First, Figure 1 specifies the numerical values of time (t) corresponding to Temperature (T) at different time in our universe's evolution.<3><4><2>

Second, Formulas (1a) below describes our universe's evolution relevant between Radiation Era and Big Bang in Figure (1), (from t = 10^{-43} s to t = 1/3×10^6 years).<3><4><

$Tt^{1/2} = k_1$, <4><6>, $R = k_2 t^{1/2}$, $RT = k_3$, $R = k_4 \lambda$ **(1α)**

t—Characteristic Expansion Time, T—Temperature of Radiations, R—Characteristic Size or Dimension of the Universe, λ--Wavelength of Radiation, k_1, k_2, k_3, k_4—Constants,

Formula (1b) below describes our universe's evolution relevant within the Matter-Dominated Era in Figure 1, (from t = 1/3 ×10^6 years to the present).<3><4><6>

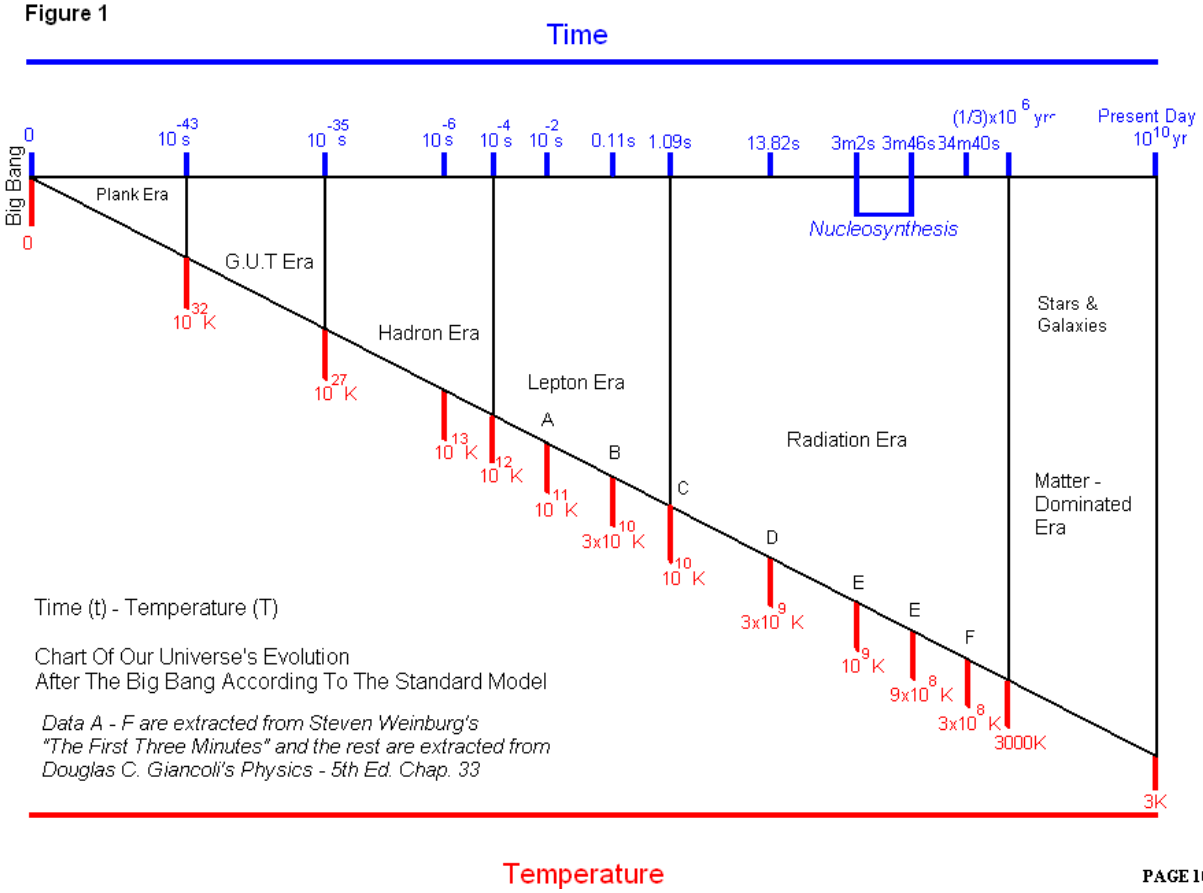
$Tt^{2/3} = k_6$, <4><6> $R = k_7 t^{2/3}$, $RT = k_8$, $R = k_9 \lambda$ **(1b)**

t – Characteristic Expansion Time, T – Temperature of the Radiation, R – Characteristic Size or Dimension of the Universe, λ – Wavelength of the Radiation, k_6, k_7, k_8, k_9 – Constants

$R = k_2 t^{1/2}$ in Formulas (1a) and $R = k_7 t^{2/3}$ in (1b) conform to Cosmological principle, GTR, Newton's Mechanics and modern observations, and can be derived from the law of energy conservation ($V^2/2 = GM/R$). (See appendix of "The First Three Minutes", by S. Weinberg and 9th paragraph behind).

The numerical values of Figure 1 above and the calculated results from Formulas (1a) and (1b) are put on Chart 1 of Appendix A for comparison, both can almost obtain the same numerical values or the same results. Those values of the universe's evolution are the sources from different theories and different calculating methods. It confirms that the laws (1a) and (1b) of our universe's evolution are reliable and correct.

Figure 1



If a group of special numerical values taken from Appendix A replace k_n, Formulas (1a) and (1b) can be used to calculate out the values of some other physical parameters.

For example, in Matter-Dominated Era, the numerical values below calculated out from Formula (1b) accord with the values on Figure 1 above and Chart 1 on Appendix A behind.

$R_1/R_2 = (t_1/t_2)^{2/3}$, $^{<3><4><6>}$ $R_1T_1 = R_2T_2$, $R_1/R_2 = \lambda_1/\lambda_2$,

When $t_1 = (13 \times 10^9$ yr) to $t_2 = (4.0 \times 10^5$ yr), $t_1/t_2 \approx 32{,}500$, $(t_1/t_2)^{2/3} \approx 1{,}000$

$R_1/R_2 = (12 \times 10^{27}\text{cm}) / (12 \times 10^{24}\text{cm}) \approx 1{,}000$

$T_1/T_2 = 3\text{K} / 3{,}000\text{K} \approx 1/1{,}000$, $\lambda_1/\lambda_2 = 0.1\text{cm} / 10^{-4}\text{cm} \approx 1{,}000$,

From the beginnings of the Matter-Dominated Era to the present, the numerical values show that, as time (t) in the universe's evolution enlarged 32,500 times, its size (R) enlarged 1,000 times, its radiant temperature (T) decreased 1,000 times, and wavelength (λ) of radiation increased 1,000 times. The results above are consistent with the modern observations and MBR (Microwave Background Radiation).

The size of our newborn universe might be like a grain of "Grape". That "Grape" might come from two ways. One way to come from Singularity is impossible. Another way is that "Grape" could come from the Big Crunch of pre-universe. It will be proved as below. Formulas (1a) is effective to the early expanding process of present universe, and effective to the later collapsing process of pre-universe too. Those two processes were all under the action of the sole gravity produced by the total energy-matters of the universe and were all in an isolated system.

2. In Newborn-hood Universe, the Properties of Radiations and Particles in the States of Super High Temperature

Quantum Mechanics (QM) indicates that all matters and radiations have the duality of particles and waves. In earliest universe, particles and radiations had same grade of ultra-high energy and could transfer between each other. Three formulas of energy below are equally valid for particles and radiations in the state of the ultra-high temperature;

$E = mc^2$, $E = \kappa T$, $E = Ch/\lambda$ (2a)

$m = \kappa T/C^2$, $\lambda \propto R$, $R \propto 1/T$ (2b)

E – Energy of single particle or radiation. m - Mass of particle, C- Light Speedκ- Boltzmann's Constant,C-Light Speed, κ- Boltzmann's Constant, h - Plank's Constant , λ - Wavelength of Radiation

Therefore, In early universe, when Temperature (T) became higher than the valve temperature of some particles, those particles would become a state of thermodynamic equilibrium with the radiation and would not stop transforming between each others. That demonstrates no differences between radiations and particles. Thus, Formulas (2a) and (2b) was effectively applied in early universe.

Formula (2b) indicates that **when the particle's Temperature (T) goes up, its Mass (m) will correspondingly increase proportionally. Inevitably, it leads to the increase in gravitational force between two closest particles.** That shows exactly why pre-universe could not stop to contract its volume (R) to a cosmic "Grape" or the Big Crunch.

3. The Condition Occurred from Big Crunch of Pre-universe to Big Explosion (Big Bang) of Present Universe

From the formulas (1a) $R = k_2 t^{1/2}$, when pre-universe contracted its size (R) to the Big Crunch, correspondingly its Temperature (T) and Mass (M) would increase, and its time (t) would too much shorten. At an extreme circumstance, when (R) contracted to such an infinitesimal dimension, the real distance between the two neighboring particles would finally become greater than the product of C (light speed) multiplied by t (time). It shows that there would not be enough time to transmit the gravity between neighboring particles. At that moment, all adjacent particles would instantaneously break off the linkage of gravitational forces and lead the pre-universe to stop contraction. Thus, the pre-universe would change its state from the Big Crunch to the Big Explosion of the present universe. That is scientifically better known as "phase transition." Such a process is different with the Big Bang as an infinitesimal explosive point of Singularity known by most individuals. In reality, Big Crunch was just a big contraction; Big Bang was just a big expansion.

Of course, the detailed process of changing states should be extremely complicated**. Once the expansion of the present universe steadily took place, due to that the increase in size (R) was much less than the increase in time (t), the gravitational force of the two closest particles would recur and renew to connect them together.** Subsequently, our universe would begin a completely new process of uniform expansion until present.

The transitive condition occurred from the Big Crunch of pre-universe to the Big Bang of the present universe is demonstrated by Formula (3) below:

$$\mathbf{dm > |\, t|\, C \quad or \quad -d_m \le C \times t \le d_m \quad or \quad -d_m / C \le t \;\&\; t \le d_m / C} \qquad \mathbf{(3)}$$

t – Characteristic Expansion Time, dm – Distance between Two Closest Particle

C – Light Speed

Let ρ = energy-matter density g/cm^3, H = Hubble's Constant

$\rho dm^3 = m \qquad m = \kappa T/c^2 \qquad \therefore t^3 < (\kappa T)/(\rho c^5)$ **(3a)**

$\rho = \underline{3H^2/(8\pi G)} = \underline{3/(8\pi G t^2)}$ <3> $\qquad \therefore t < T(8\pi G\kappa)/(3C^5)$ **(3b)**

From (1a) $Tt^{(1/2)} = k_1 \qquad \therefore t^{(3/2)} < k_1(8\pi G\kappa)/(3C^5)$ **(3c)**

Formulas (3a), (3b), (3c) are all derived from Formula (3), and have the same value of (t). They accord with the principles of GTR.

Now the numerical value of (t) can be calculated as below.

First, select k_1 in Formula (1a) from column (C) of Chart 1 on Appendix A behind,

t = 1.09 s, T = 10^{10} k. [the same results can be gotten by other than column (C)]

$k_1 = Tt^{(1/2)} = (10^{10}) \times (1.09s)^{(1/2)} \approx 10^{10}$ (in some books, $T \times t^{1/2} \approx 10^{10}$ may be as a experiential formula),

From Formula (3c), $t^{(3/2)} < [(8\pi G\kappa)k_1 / (3C^5)]$

$G = 6.67 \times 10^{-8}$ (cm^3/gs^2), $\kappa = 1.38 \times 10^{-23}$ J/k = 1.38×10^{-16} gcm^2/s^{2}k, $C = 3 \times 10^{10}$ cm/s

$t^{3/2} \le [8\pi(6.67\times10^{-8})(1.38\times10^{-16})(10^{10})] / [3(3\times10^{10})^5] = 0.32\times10^{-64}$

$\therefore\ \mathbf{t \le +10^{-43}s}$ **and** $\mathbf{t \ge -10^{-43}s}$ **(3d)**

Corresponding $T = k_1/t^{(1/2)} = 10^{10}/(10^{-43})^{1/2} = 0.32 \times 10^{32}$k, $\mathbf{T \approx 0.32 \times 10^{32}\ k}$ **(3e)**

From Formula (2b), mass of particle, $m = \kappa T/C^2 = 10^{-5}$g, $m = 0.5\times10^{-5}$g.

$\rho = 3/(8\pi Gt^2) \approx 1.8\times10^{92}$ g/cm^3, $d_m{}^3 = m/\rho$, so, $d_m \approx 14\times10^{-33}$ cm, $C\times t = 3\times10^{10}\times 10^{-43} = 3\times10^{-33}$ cm.

So, $\mathbf{d_m > C \times t.}$ **(3f)**

Thus, **the gravities of closest particles had surely broken off at time of ($\mathbf{t = -10^{-43}}$ s).**

The calculated values $t \ge -10^{-43}$s, $t \le +10^{-43}$s, and $T \approx 0.32 \times 10^{32}$k are precisely in accordance with the values at the beginning or ending of Plank's Era on Figure (1).

The calculations shows that once the Big Crunch of pre-universe contracted to $t = -10^{-43}$ s and $T = 0.32 \times 10^{32}$ k, the gravity connected to the two closest particles would thus disappear. **No gravity is equal to no power for contraction, and then $T \approx 10^{32}$ k become the**

highest temperature in Universe. With no gravity, the only way for the pre-universe and for particles was to stop their contraction and then started the expansion to the present universe at the highest temperature of $T \approx 10^{32}$ k. After that, $t = +10^{-43}$ s would become the time required for recovering the gravitational linkage between two neighboring particles at the genesis of our present expanded universe.

Between $t = -10^{-43}$ s and $t = +10^{-43}$ s, there should be appearance of time (t = 0). However, **time (t = 0) does not signify the presence of Singularity at all,** since at that point of (t = 0), the temperature $T \approx 10^{32}$ k, T was not infinity. The density $\rho \approx 10^{92}$g/cm$^3 \neq 0$, and the actual radius of universe $R \neq 0$. So, **the point of (t = 0) is just a bridge between contracted state ($t = -10^{-43}$ s, +R) and expanded state ($t = +10^{-43}$ s, +R).**

4. Quantum Micro Black Holes (QMBHs)

From Formulas (3) and (3c) of paragraph 3, once pre-universe collapsed to ($t = -10^{-43}$ s, $T = 10^{32}$ k), the gravitational linkage between the closest particles would break off. At that moment, the mass of any particle or radiation m_t is gotten from (2b),

$$m_t = \kappa T / C^2 = 1.38\times10^{-16}\times10^{32}/(3\times10^{10})^2 = 1.5\times10^{-5}\ \text{g} \quad (4a)$$

From (3b), $\rho_t = 3 / (8\pi G t^2) \approx 7\times10^{92}$g /cm^3 , so, radius r_t of m_t,

$$r_t = (3m_t / 4\pi\rho_t)^{1/3} = 1.4\times10^{-33}\ \text{cm} \quad (4b)$$

If each particle ($m_t = 10^{-5}$g) is as a QMBH, its Schwarzchild's radius r_b is:

$$r_b = 2Gm_t / C^{2\ <3>} = 2\times6.67\times10^{-8}/(3\times10)^2 = 1.48\times10^{-33}\text{cm} \quad (4c)$$

So, **$r_b = r_t = 1.5\times10^{-33}$cm, and ($m_t \approx 10^{-5}$g) is a exact micro black hole.**

Formula (4c) has proved that ($m_t = 10^{-5}$ g) is a sure QMBH at the state ($t = -10^{-43}$s, $T = 10^{32}$ k). Due to the density ρ_t of ($m_t = 10^{-5}$g), $\rho_t \approx 10^{93}$g/cm$^3 >> 10^{15}$ g/cm^3 (density of a neutron broken up). Thus, m_t as a micro black hole was composed by the perfectly micro quantum. The temperature T_b of Hawking's formula of m_t as a QMBH is: (M_θ - Mass of sun)

$$T_b \approx 0.4\times10^{-6}\, M_\theta / m_t = 0.5\times10^{32}\text{k},\ ^{<5>} \quad (4d)$$

$$T_b = T \approx 10^{32}\text{k} \quad (4e)$$

Formula (4e) shows that any particle m_t is a perfect QMBH.

The lifetime τ_b of Hawking's formula of the QMBH m_t is: $\tau_b \approx 10^{-27} m_t^3 \approx 10^{-43}$s $^{<5>}$ (4f)

τ_b was consistent with the time which pre-universe collapsed from ($t = -10^{-43}$s) to (t = 0), or **QMBHs would disappear simultaneously with the disappearance of pre-universe in the Quantum Field.** Then, the genesis of our universe came out from the Quantum Field in Plank's Era, but not Singularity.

$$t_b \times r_b = 0.5\times10^{32}\text{k}\times1.5\times10^{-33}\text{cm} \approx 0.075\text{cmk} \quad (4g)$$

Formula (4g) indicates that the property of QMBHs accords with the Uncertainty Principle of Quantum Mechanics.

5. Uncertainty Principle of QM Was Applied to Quantum Gravitation $^{<6>}$

According to the Uncertainty Principle of QM, (Quantum Mechanics)

$$\Delta E_* \times \Delta t \approx h/2\pi \quad (5a)$$

$h = 6.625\times10^{-27}$ erg s, h – Plank's constant. Applying formula (5a) to the reactional process of two elementary particles, $\Delta E = 2mC^2$ (5b)

Δt is the time producing or annihilating two particles (m – mass of particle), $\Delta t = t_c = h/(4\pi mC^2)$ (5c)

t_c – Compton time. t_s – Schwarzchild's time, i. e. the time of light passing through the Schwarzchild's radius of particle. $t_s = 2Gm/C^3$ (5d)

Generally, $t_c < t_s$, in case of $t_c = t_s$, then $m = m_p$, m_p - Plank;s mass, $m_p = (hC/8\pi G)^{1/2} = 10^{-5}$g (5e)

According to Uncertainty Principle, time t_p is corresponding to m_p,

$$t_p = (Gh/2\pi C^5)^{1/2} = 0.539\times10^{-43}\text{s} \quad (5f)$$

t_p is called as Plank's time, l_p is Plank's length corresponding to t_p, temperature T,

$$l_p = t_p \times C = (Gh/2\pi C^3)^{1/2} = 1.6\times10^{-33}\text{cm} \quad (5g)$$

$$T = m_p \times C^2/\kappa = 0.65\times10^{32}\text{k} = 10^{19}\text{GeV} \quad (5h)$$

When the universal age was less than the Plank's time t_p, the quantum effect would appear, time might not be measured precisely.

Plank's time ($+t_p$) only has the positive value in original meaning, the new concept above shows that **the negative value ($-t_p$) has the meaning of time too, at time ($-t_p$), pre-universe collapsed to lose gravity between the closest particles and stopped collapsing.**

Checking up the numerical values above, the results are compared as below:

No Linkage of Gravity	QMBH	UPQM
$t = -10^{-43}$s & 10^{-43}s ,	$\tau_b = 10^{-43}$s,	$t_p \approx 0.5\times10^{-43}$s,
$T \approx 0.32\times10^{32}$k,	$T = 0.5\times10^{32}$k ,	$T = 0.65\times10^{32}$ k,
$m \approx 0.5\times10^{-5}$ g,	$m_t \approx 1.5\times10^{-5}$ g,	$m_p = 10^{-5}$ g,
$d_m/2 \approx 7\times10^{-33}$ cm,	$r_b \approx 1.5\times10^{-33}$cm,	$l_p \approx 1.6\times10^{-33}$ cm,

6. Reviews to Our Present Universe

Our present universe looks like a gigantic black hole. If the age of our universe is

$L_u = 140\times10^8$years, its Schwarzchild's radius $R_u = L_u \times C$.

The total mass inside the Event Horizon of our universe: $M_u = C^3 L_u / 2G \approx 10^{56}\text{g} \approx 10^{23} M_\theta$,

The radius r_o (before "Inflation") of M_u at the genesis of original Universe;

$r_o = (3M_u/4\ \pi\ \rho)^{1/3} = (3\times10^{56}/4\ \pi\ \times10^{93})^{1/3} = 1.3\times10^{-12}$cm.

The size of original Universe of M_u looks like the size of a present proton or a neutron.

The numbers of particles or QMBHs of M_u in the original Universe are;

$N_o=M_n/m_t=10^{56}/10^{-5}=10^{61}$

The proton numbers of M_u of original Universe are;

$N_{op}=M_u/m_{proton}=10^{56}/1.67\times10^{-24}\approx 10^{80}$.

Mankind lives in the gigantic universal black hole, a great number of small and big black holes have scattered in the boundless space.

7. Conclusion: The origin and process turned from the disappearance of pre-universe to the birth of present universe in Plank's Era ($-10^{-43}s \le t \le +10^{-43}s$)

(A) The transitive origin caused from the big contraction of pre-universe to the big expansion of present universe

The calculated results above show that, once pre-universe collapsed to $t = -10^{-43}$s and then began to make a "phase transition" from the past contracted universe to the present expanding universe**. From new formula (3c) $t^{3/2} \le k_1(8\pi G\kappa)/(3C^5)$, value of ($t = 10^{-43}$s) can be exactly calculated out.** In the extremely short interval of time ($-10^{-43}s \le t \le +10^{-43}$), every particle and radiation in whole universe were in micro-quantum field of Plank's Era and would become a QMBH ($m_t=10^{-5}$g, $r_b=10^{-33}$cm, $T=10^{32}$k). They simultaneously entered three states: **no gravitational linkages between the closest particles, quantization and micro black holes (which were all at the explosive state) (see 19th paragraph of part two behind). Such three states of particles and radiations jointly obstructed the appearance of Singularity in the process of the big contraction of pre-universe, and then led the genesis of our present Universe from new QMBHs. That is one of the new concepts in this article.** Each of the physical parameters in the three states above had the equivalent numerical values at the same time. Those numerical values derived from many current classical theories can successfully reach the same results. **The important contribution in this article is to have found out QMBHs ($m_t \approx 10^{-5}$g).** Only the process of such a "phase transition" at the birth of our universe can exactly accord with the causality, the second law of thermodynamics and the law of energy conservation. Singularity possessed some infinite physical amounts has no possibility to become a stable being inside and outside, so it cannot appear and exist in universe.

(B) The gradual vanish of pre-universe in the interval of time ($-10^{-43}s \le t$) $\Rightarrow$ ($t \approx 0$) in Plank's Era

Once the past universe collapsed to the time ($t \ge -10^{-43}$s) of Plank's Era, every particle became an isolate QMBH of ($m_t \approx 10^{-5}$g) and had no gravity between each other. Gravitational transmissions between two closest particles needed time 10^{-43}s or more. Thus, **all MQBHs had no enough time to attain combination, but could only emit Hawking Radiations until gradually thorough disappearance within time 10^{-43}s i.e. ($-10^{-43}s$)$\Rightarrow$($t \approx 0$). The completely pre-universe would synchronous disappear too.** The disappeared process of a single MQBH at the super-high temperature might be a small bang like a double-bang firecracker, and the disappeared process of completely pre-universe included countless MQBHs might be like a great lump of beautiful firework. The vanish of the old universe in quantum field would not hand down any information or trace of pre-universe to present universe.

According to definition of GTR, formulas of a Schwarzchild's BH are:

$$C^2/2 = Gm_b/r_b, \text{ or } C^2 r_b/(2G) = m_b, \quad C\times t = r_b, \quad (7a)$$

m_b –mass of BH, r_b–Schwarzchild's radius of BH, ρ_b–density of BH, for a spherical BH,

$$m_b = 4\pi\rho_b r_b^3/3 , \quad (7b)$$

From (7a), (7b),

$$t^2 = 3/(8\pi G\rho_b) \quad (7c)$$

Formula (7c) indicates that a certain density of BH ρ_b is only corresponding to a sole time t (t-time of light passing through BH). In other words, **for a steady BH, once a parameter such as m_b is decided as a certainly numerical value, all other parameters of BH (r_b,t, t_b,ρ_b and etc.) will be solely decided with the first one. That is the essential quality of any BH**. In Plank's Era, $\rho_b \approx 1.8\times10^{92}$ g/cm^3 $\approx$ constant, as a result, $t = 10^{-43}$ s, $m_b \approx 1.5\times10^{-5}$ g, $r_b \approx 1.5\times10^{-33}$ cm, $t_b \approx 10^{32}$ k.

It is said, under the condition of $\rho_b \approx 10^{92}$g/cm^3, time (t) of forming a BH ($m_b \approx 10^{-5}$ g) must need $t \ge 10^{-43}$ s. In case of $t < 10^{-43}$ s, if some new particles (its mass $m << 10^{-5}$ g) formed in this interval of time, they would not become smaller QMBHs at all, because a smaller BH of ($m_b << 10^{-5}$ g) must be formed by the greater density ($\rho_b >> 10^{92}$ g/cm3).

(C) The genesis of our new universe within Plank's Era of ($t \approx 0$)$\Rightarrow$($t \le +10^{-43}$ s)

The mini embryos of particles($m << 10^{-5}$g) might be formed at first, but they should have enough time growing up to QMBHs of ($m_b \approx 10^{-5}$g) within this interval of 10^{-43}s. However, in these two intervals of sections (C) and (B), the universal temperature (T, t_b) and density (ρ_b) should lower a little bit expansion at ultra-high temperature with no gravity. Thus, the **new QMBHs (or particles)** re-combined possibly from micro quantum should have a little bigger size: $m_{bn} > 10^{-5}$g, $r_{bn} > 10^{-33}$cm, $t_n > 10^{-43}$s. It is said, in case of time (t) reached 10^{-43}s, there would not be enough time for particles to grow up to the complete bigger QMBHs ($m_b > 10^{-5}$g). Particles had to become bigger QMBHs only with the time prolonged over ($t > 10^{-43}$s). Thus, the bigger QMBHs ($m_b > 10^{-5}$g) could only compose and re-combine or collide out of Plank's Era ($t > 10^{-43}$s) i. e. into GUT Era. That was the origin of "Primordial Inflation" at the birth of our present universe. However, particles grown up to mass $\approx 10^{-5}$g

would not be BHs due to temperature and density lowered a little bit.

(D) "Inflation Era" of the primordial universe ($t > +10^{-43}s$)⇒ GUT Era

Owing to the decrease in the universal density ρ_b, the little bigger QMBHs might form into GUT Era of ($t > 10^{-43}s$), due to its formed time to be prolonged. According to the principle of BH, if a new BH was formed from the collision of two old BHs, the properties of new BH are as behind: Suppose parameters of old BH 1; $C^2/2 = GM_1/R_1$, old BH 2; $C^2/2 = GM_2/R_2$,

M,R,-parameters of new BH.

Due to $M = M_1 + M_2$, as a result; $R = R_1 + R_2$. That is the origin of "Inflation".

Above formula clearly indicates: a), the collision of two or more BHs would certainly cause "Inflation". b) "Inflation" caused in GUT Era . c) A new BH formed from the collisions of two or more old BHs was a real and a complete bigger BH.

Every small explosion caused from collisions between adjacent QMBHs at every point of newborn universe would compose jointly a greatest burst (i.e. so-called Big Bang) in whole universe. However, those explosions were not a suddenly strong burst at one point like so-called "Big Bang" of Singularity, but certainly caused "Primordial Inflation" of newborn universe from GUT Era. That was the parturient pangs and the real birth of our present new universe. After collisions, QMBHs would combine and merge for a very long time until becoming a new perfect BH. That was an extremely long expanded process.

(E) The reason for nonstop expansion of our universe until present

Let us look back to formula (7c), $t^2 = 3/(8\pi G\rho_b)$. Formula (7c) is derived from principles of BH, (t) is the time of that, light (C) passes through Schwarzchild's radius (r_b) of a BH. However, formula (7c) can express our present universal expanding law, if density of BH ρ_b is replaced by our universal real density ρ. As a result, $t^2 = 3/(8\pi G\rho)$. Here(t) would express the age of our universe. It is the complete same with formula (3b), which is easily derived from famous Hubble's law (V=HR, H = 1/t) and from the law of energy conservation ($V^2/2 = GM/R$, and $M = 4\pi\rho R^3/3$) in the universal evolution. **It shows in a sufficient expanded universe, its boundary would combine with Event Horizon of sufficient expanded universal BH, and then (V=C).**

(3b) ≅ (7c) **(7d)**

Check up the calculated numerical values: At the birth of our universe, for a QMBH, its mass $m_t \approx 10^{-5}$g, its Schwarzchild's radius $r_b \approx 1.5\times10^{-33}$cm. If our present universal BH is surely composed from the combinations of "primordial inflation" of QMBHs at the birth of universe, hence, the radius of Event Horizon of present universe R_u must be equal to the total sum of the radius of primordial QMBHs (r_b). From 6th paragraph, the mass of present universe within Event Horizon $M_u \approx 10^{56}$g, the numbers of primordial particles (QMBHs) corresponding to M_u are: $N_o \approx 10^{61}$.

As a result: $M_u = m_t \times N_o = 10^{-5}\times10^{61} \approx 10^{56}$g, $R_u = r_b \times N_o = 1.5\times10^{-33}\times10^{61} \approx 1.5\times10^{28}$cm. Checking the real size (R) of present universe on Appendix A (Chart 1) behind,

$R \approx 12\times10^{27}$cm, so, **$R = R_u \approx 10^{28}$cm** **(7e)**

Therefore, the present expansion of our universe did surely come from the collisions or combinations(primordial inflation) of countless QMBHs from GUT Era to later time.

(7e) shows that, our present universe is a perfect gigantic BH expanded from $N_o \times m_t$.

(F) Whether or not expansion of our universe at present would not depend on the universal real density ρ, but only depend on the total energy of primordial universal packet M_o, the end of our universe

From (7c), (7d), (7e), it will be seen that, for a steady BH (mass = M_b), only a sole density ρ_b can correspond with certain M_b. Certainly, $\rho = \rho_b$ is all right for any BH. That is the character of BHs. Obviously, **$\rho_b = \rho = \rho_c$, or $\Omega = \rho/\rho_c = 1$, that is an inevitable outcome to our sufficient expanded universe as well as a sufficient expanded BH**. Therefore, in several decades ago, the debates or researches about ($\Omega \neq 1$ or $\Omega = 1$) seemingly had no significance.

Now let us review how to get mass M_u of our universe within its Event Horizon, firstly, real density $\rho \approx 2\times10^{-29}$ g/cm^{3}could be measured, then, from formula (3b), $t^2 = 3/(8\pi G\rho)$, t is the age of our universe, $t \approx 4.23\times10^{17}$ s $\approx 1.35\times10^{10}$ yrs. Thus, the radius of Event Horizon $R_u = t\times C = 1.2\times10^{28}$cm, and $M_u = C^2R_u/(2G) \approx 10^{56}$g $\approx 10^{23}\ M_\theta$. Since our universe is a real BH, a certain M_u should correspond with a certain and sole ρ. Thus, a deduction below should be gotten. If there is more mass ($M_o - M_u$) outside Event Horizon, M_o – total mass of our originally universal packet, M_u will increase as the enlargement of Event Horizon. Only under the condition of $M_o - M_u = 0$, or $M_o = M_u$, our universe will stop to expand. After that, our universe will only show to emit Hawking's radiation, to lose energy-matters and to contract its size till thorough disappearance. The lifetime of disappearance (τ_o) is extremely long, (τ_o) can be calculated by formula (4f), $\tau_o \approx 10^{-27}M_o^{3}{}_{(s)}$. For example, if $M_o = M_u \approx 10^{23}M_\theta \approx 10^{56}$g, $\tau_{ou} \approx 10^{130}$ yrs.

(G) Several years ago, some astrophysicists proposed that our universe is accelerating its expansion according to observations from remote supernova 1a, and pointed out that over 60% dark energy of exclusive force exists in universe[1]. Since our universe as a gigantic BH should inevitably possess the essential quality of any

BH, the expansion of our universe only depends upon absorbed energy-matters which did not belong to our original universe. More energy-matters were taken in, faster its expansion would be. Of course, if absorbed energy possesses exclusive force, the expansion of universe should be much faster or accelerated.

8. The further explanations

The new concepts in this article show that GTR cannot be applied to describe the state of quantum field at Plank's Era (-10^{-43}s) $\Rightarrow 0 \Rightarrow (+10^{-43}$ s) in the primordial universal evolution, just as Newton's mechanics cannot describe the motion of any matter which speed is close to the light speed (C). Without exception, the mathematical equations of all theories including GTR would always have its applied conditions and limits.

The four difficult and complicated problems (Singularity, flatness, Event Horizon and magnetic monopole) at the genesis of universe had troubled scientists for several decades. After existence of Singularity has been negated by new concepts in this article, the other three problems may be easily solved. Moreover, the new concepts in this article have given the better explanation to the origin of "Inflation" and "inflationary cosmological model".

If the new concepts in this article exclude the occurrence and existence of Singularity at the genesis of our universe, for which scientists will not need to beg the marvels or any new theories or to provide some special original conditions.

All numerical values calculated in this article are precisely consistent with the current classical theories and its formulas, the observational results and the real evolutionary process of our universe. Probably, the new concepts in this article may not be accepted and convinced by the most scientists because of no abstruse theory, no complicated mathematical equations, no exact demonstrations as well as the old conventions broken down.

9. Demonstration to formulas (1a) and (1b)

To prove the Formulas (1a, 1b) as below, Suppose R is the radius of a sphere in the universe. R's dimension should be large enough.

The potential energy (P.E.) on the spherical surface is:

P.E. = (mMG)/R,

m – Mass of a particle on the surface,

M – Total masses in the sphere of radius R

G – Gravitational Constant,

R – Characteristic scale factor (dimension)

The kinetic energy (K.E.) of m on the surface is

K.E. = $0.5\ mV^2$.

V–Radical Velocity, expanding or escaping velocity are corresponding to the end-point of R.

According to the "Cosmological Principle", the universe, which is both isotropic and homogeneous, looks the same in any direction and at every point. Therefore, the whole sphere should be considered an isolated system and no energy exchange with the other system. Based on the principle's of Newton Mechanics, the real space with three dimensions can be treated in one dimension R in the process of the universe's evolution.

P.E. = K.E.

$0.5V^2=(MG)/R$ (B)

(1) In the process of the universe's evolution from Big Bang to the end of Radiation Era, to particles or radiations (photons), from (2a), (2b), (1a)

$E=mc^2$ $E=\kappa T$ $E=ch/\lambda$ (2a)

$m = \kappa T/C^2$, $R \propto \lambda$, and $T \propto 1/R$, (2b)

Formulas (2a), (2b) show that, in the early process of universe's expansion, the increase in wavelength of the radiations λ is proportional to increase of R, and **as temperature T slow down, mass m of a particle would decrease, and then the gravity between particles would weaken. That is the important reason for that, pre-universe quickened its contraction to Big Crunch and the present universe accelerated its expansion in the earlier period after its birth.**

Because in Formula (B), $\Sigma m_i = M$ (B1)

Therefore, M's increasing is proportional every m_i's increasing,

From Formula (B)(B1) (2b),

So $M \propto 1/R$ **$M \neq$ Constant** (B2)

From Formula (B)(B2), $V^2 \propto 1/R^2$, $V \propto 1/R$, VR=Constant

So RV = Constant i.e. $R(dR/dt)$ = Constant (B3)

From (B)(B1)(B2)(B3) , dR^2/dt = constant

$Tt^{1/2} = k_1$, $R=k_2t^{1/2}$, $RT = k_3$, $R = k_4\lambda$, $RV = k_5$ (1a)

Formula (1a) is proven correctly.

(2). In The Matter-Dominated Era

Because in Formula (B), **M = Constant**

So $R^{1/2}V$=Constant i.e. $R^{1/2}(dR/dt)$ = Constant, So $R^{1/2}V$=Constant

Therefore, $R^{3/2} = t \times$(Constant), $R = k_7 t^{2/3}$ (B4)

In this era, to radiation, Formula (B1) is still right

So from Formulas (B1)(B4)

$Tt^{2/3} = k_6$, $R = k_7t^{2/3}$, $RT = k_8$, $R=k_9\lambda$, $R^{1/2}V = k_{10}$ (1b)

Formula (1b) is proved correctly.

10. Appendix A

Chart 1. Values Compared Between Figure 1 And Formulas (1a) (1b)

10. Appendix A

Chart 1 Values Compared Between Figure 1 And Formulas (1a) (1b)

	I Matter-Dominated Era		II Radiation Era					III Lepton Era	
Time (t) yrs,min,sec Figure 1	13.0×10^{9} yrs	4×10^{5} yrs	***F*** 34 m 40 s	***E*** 3 m 46 s	***E*** 3 m 2 s	***D*** 13.82 s	***C*** 1.09 s	***B*** 0.11 s	***A*** 2×10^{-2} s
Temperature (T) K Figure 1	3 K	3000 K	3×10^{8} K	9×10^{8} K	10^{9} K	3×10^{9} K	10^{10} K	3×10^{10} K	10^{11} K
	Formula (1-b)		***Formula (1-a)***					***Formula (1-a)***	
Temperature (T) Kelvin	3 K	3000 K	2.3×10^{8}	7.1×10^{8}	0.8×10^{9}	2.9×10^{9}	1.02×10^{10}	3.2×10^{10}	0.75×10^{11}
R cm	12×10^{27}	12×10^{24}	1.5×10^{20}	0.5×10^{20}	4.6×10^{19}	1.5×10^{19}	3.5×10^{18}	1.1×10^{18}	4.8×10^{17}
Wavelength (λ) cm	0.1	10^{-4}	1.25×10^{-9}	4.2×10^{-10}	3.8×10^{-10}	1.25×10^{-10}	2.9×10^{-11}	9×10^{-12}	4×10^{-12}
ρ_c g / Cm ^{3}	10.6×10^{-30}	1.12×10^{-20}	0.4	35	54	9.4×10^{3}	1.5×10^{6}	1.48×10^{8}	4.5×10^{9}

IV Hadron Era				V Plank Era
10^{-4} s	10^{-6} s	10^{-35} s	10^{-43}	
10^{12} K	10^{13} K	10^{27} K	10^{32} K	
Formula (1-a)				
1.07×10^{12}	1.07×10^{13}	3.38×10^{27}	0.338×10^{32}	
3.4×10^{16}	3.4×10^{15}	10.7	10.7×10^{-4}	
2.8×10^{-13}	2.8×10^{-14}	8.85×10^{-29}	8.85×10^{-33}	
1.8×10^{14}	1.8×10^{18}	1.8×10^{76}	1.8×10^{92}	

Take the initial numerical values below, which are at present universe time:
All other values on the above chart can be calculated out according to Formulas (1a, 1b)
Suppose the Hubble's Constant H = 75 Km/(sxMP_{sc})

So $t_0 = 1/H = 13 \times 10^{9}$yr

$R = t_0 c = (13 \times 10^{9}\text{yr}) \times c = 12 \times 10^{27}$cm $\quad \lambda = 0.1$ cm $\quad \rho_c = 10.6 \times 10^{-30}$ g/cm^3 $\quad T = 3$k

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Concept and Protocol to Isolate Cholesterol-reducing Bacteria from Carnivores

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Abstract: Cholesterol plays a major role in human heart health. However, the body makes enough cholesterol, so any dietary cholesterol isn't needed. High cholesterol is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke. Excess cholesterol in the bloodstream can form plaque (a thick, hard deposit) in artery walls. It is necessary for animal to have bacteria to reduce cholesterol in the blood. Lactobacillus bacteria and bifidobacteria decrease blood levels of cholesterol by either increased intestinal absorption and removal through solid excretion, or by limiting the conversion of cholesterol back into bile acids for storage in the liver. Certain bacteria have been known to degrade serum cholesterol in gut of animals. Carnivores (predators) normally eat meat containing high fat and rarely develop atherosclerosis or have cardiovascular events. It is possible to select and characterize strains of bacteria from carnivores that remove cholesterol from blood serum or control blood serum cholesterol level. [Nature and Science. 2004;2(4): 11-17].

Keywords: animal; bacteria; cholesterol; cardiovascular disease; carnivore (predator)

1. Introduction

Cholesterol plays a major role in human cardiovascular pathogenesis. Normally it is needed in the body to insulate nerves, make cell membranes and produce certain hormones. However, the body makes enough cholesterol, so dietary cholesterol intake is not essential for normal adult metabolism. High serum cholesterol level is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke - America's number one killer (Tabas, 2002). Excess cholesterol in the bloodstream can form plaque in arterial walls. The cholesterol and plaque build-up causes the arteries to become thicker, harder and less flexible, slowing down or/and sometimes blocking blood flow to the heart and other vital organs. When blood flow is restricted, angina (chest pain) can result. A heart attack will result when blood flow to the heart is severely impaired and a clot stops blood flow completely. When too much low-density lipoprotein (LDL) deposits inside the arterial walls, where if is is oxidized, it can build up as hard deposits and cause atherosclerosis, the disease process that underlies heart attacks. There are 102.3 million American adults who have total blood cholesterol values of 200 mg/dl and higher, and about 41.3 million American adults have levels of 240 mg/dl of cholesterol or above. The control of serum cholesterol is the major concern of the modern medicine. Oxidation is important relevant to atheroscelrosis (Steinberg, 2002). However, certain types of bacteria have the ability to inhibit LDL oxidation and, thus, attenuate atherogenesis (Lavy, 2004; Kullisaar, 2003; Terahara, 2000; Terahara, 2001).

Lactobacillus bacteria and bifidobacteria in the host decrease blood cholesterol levels by either decreased total intestinal absorption or removal through solid excretion, or by interrupting the entero-hepatic cycle of bile acids (James, 1999). Investigation at the Shinshu University in Japan demonstrated that *Lactobacillus acidophilus* bacteria suppressed the re-absorption of bile acids carrying cholesterol and enhanced the removal of cholesterol from blood through faeces (Hosono, 2000). Another study demonstrated that increased lactobacillus bacteria in intestine lowered total blood cholesterol by 22% and triglycerides by 33% (Taranto, 1999). Yet, another study demonstrated that lactobacillus bacteria significantly lowered blood pressure in men and women 18 to 55 years of age after eight weeks of supplementation (Agerholm-Larsen, 2000). Subjects in the control group who did not receive the selected strains of lactobacillus bacteria did not experience a drop in their high blood pressure. Therefore, there is significant evidence that specific types of lactobacillus bacteria and bifidobacteria can lower the

three major risk factors for coronary heart disease and stroke: excessive cholesterol, high blood pressure, and high triglyceride levels. However, the cholesterol reducing bacteria have to be carefully selected to achieve the best results. Not all strains of *Lactobacillus acidophilus, Bifidobacteria bifidum*, and *Lactobacillus bulgaricus* bacteria available will lower cholesterol.

Lactobacillus acidophilus occurs naturally in the human intestinal tract. Hundreds of strains may exist, and the wide degree of variation among strains is an important characteristic in determining their potential for nutrition and health benefits. These benefits include the digestion of lactose (milk sugar) as well as reducing serum cholesterol. *Lactobacillus acidophilus* Gilliland was one of the more effective strains at reducing serum cholesterol from among 123 new isolates tested (Gilliland, 2004). These investigators found two mechanisms that intestinal bacteria can lower serum cholesterol: first, as the bacteria grow in the intestinal tract, they consume cholesterol; second, at least part of the cholesterol actually becomes incorporated into the bacterial cells. Either way, the cholesterol becomes unavailable for absorption from the gut. Numerous studies, however, have demonstrated that *Lactobacillus acidophilus*, *Bifidobacteria bifidum*, and *Lactobacillus bulgaricus* bacteria in animal gut significantly lower cholesterol.

Adjustment of cholesterol level is critical for prevention of cardiovascular events. Carnivores (predators) normally eat meat containing high fat and rarely develop atherosclerosis or have cardiovascular events. If the elevated cholesterol problem in omnivores is linked to a high fat diet, introducing the beneficial bacteria into the omnivorous intestine as a supplement may offer a natural way lower cholesterol levels. There are numerous studies on the cholesterol reducing effects of bacteria in omnivores, but few reports describe cholesterol lowering in carnivores. It is very possible to isolate and characterize bacteria with cholesterol reducing ability in carnivores. These can be tested in an atherosclerotic rabbit model of plaque disruption and thrombosis. This article gives the ideas and methods to do that as a reference for anybody who is interested in this trial.

2. Material and Methods

The purpose of this article is to offer the ideas and methods to isolate and characterize specific strains of bacteria from carnivores that have the ability to reduce cholesterol from the serum of atherosclerotic and hyperlipidemic rabbits. The steps could be:

1. To isolate and culture the bacteria from carnivores (such as tiger, lion and dog) feces or intestine and to screen the specific bacteria strains that reduces cholesterol in serum.

2. To characterize and evaluate the cholesterol-reducing candidate bacteria strains from Step 1 *in vitro*.

3. To determine the bacterial strains if they can be introduced to omnivora or herbivores (such as rabbit) orally then to get the conclusion that the bacteria to function serum cholesterol level reduction without harmful side effect.

4. To test if the bacterial strains and/or their byproducts can be used as an oral medication.

2.1 Isolation of Bacteria

Feces or intestinal contents can be obtained from tigers, lions or dogs, and immediately placed in an aerobe jar, and then transported to an anaerobe container within 20 to 30 min. A 0.5 g portion of feces or intestinal contents is serially ten fold diluted in standard medium to give a bacterial content of 10^{10}/ml. A drop of the 10^2 and 10^3 dilutions is transferred with a sealed pipette to plates and streaked for isolation. The plates and the ten serial diluted tubes are incubated at 35^{o}C. The plates are observed after 3-5 days of incubation. Colonies showing the unusual morphology are inoculated until the medium is clotted, which occurs as the cholesterol-reducing bacteria approaches 10^8/ml. The clotted cultures are streaked to cholesterol agar medium to check purity and analyzed for coprostanol by gas-liquid chromatography. If suspect colonies are not present on the initial plates, the researchers should observe the serial dilution tubes for the clotting. Since large numbers of cholesterol-reducing bacteria will be present when the medium clots, researchers can streak a drop of medium from freshly clotted tubes of the dilution set on cholesterol agar medium. Several subcultures can be made in standard cholesterol agar medium to enhance the numbers of cholesterol-reducing bacteria before streaking to cholesterol agar medium (Brinkley, 1982). The isolation procedures

and biochemical testing are performed in a modified stainless steel Blickmnan chamber under anaerobic conditions (Brinkley, 1978). The gas mixture contains 10% H_2, 10% CO_2 and 80% N_2 (Pereira and Gibson, 2002). The chamber humidity is maintained at 45% relative humidity by a mechanical condenser. The cultures are incubated at 35°C in the chamber. All media are prepared aerobically and passed into the camber at least 24 hours before use.

2.2 Growth of Bacteria

Standard technique of bacteria culture is used. Briefly, bacteria sample is dissolved in 0.3 ml LB plus tetracycline (2 mg/ml) medium, transferred into a tube containing 5 ml LB plus tetracycline (2 mg/ml) medium, kept at 37°C overnight with shaking, then the bacteria frozen at –70°C. These can be divided into several tubes before freezing.

2.3 Harvesting Bacteria

A. Streak an inoculum across one side of a plate using sterile technique. Re-sterilize an inoculating loop and streak a sample from the first streak across a fresh part of the plate, and then incubate at 37°C until colonies appear (overnight).

B. Transfer a single bacterial colony into 2 ml of LB medium containing tetracycline (2 mg/ml) in a loosely capped 15-ml tube. These should be kept at 37°C overnight in Elmer flasks with vigorous shaking to enhance bacterial growth.

C. Pour 1.5 ml of the culture into a micro-centrifuge tube. Centrifuge at 12,000 rpm for 30 seconds at 4°C in a micro-centrifuge. Store the remainder of the culture at 4°C.

D. Remove the medium by aspiration.

2.4 LB Medium (Large-Bertani Medium, per liter)

To 950 ml of deionized H_2O, add: bacto-tryptone 10 g, bacto-yeast extract 5 g, and NaCl 10 g. Shake until the solutes have dissolved. Adjust to pH 7.0 with 5 N NaOH (about 0.2 ml). Adjust the volume of the solution to 1 liter with deionized H_2O. Sterilized by autoclaving for 20 minutes in a liquid cycle at 1 kg/cm^2.

2.5 Cholesterol and Plasmalogen Requirements

All isolates are streaked on supplemented cholesterol agar medium to test their ability to grow without cholesterol and plasmalogen. Isolates that grow are repeatedly subcultured on cholesterol agar medium to ensure that cholesterol and plasmalogen in the inoculum are not responsible for the growth. All isolates are inoculated into lecithin-cholesterol medium to test for cholesterol deduction in the absence of plasmalogen. Each isolate is also inoculated into lecithin-cholesterol medium containing 0.5 mg of PLE as a positive control. Cultures which reduce cholesterol in the lecithin-cholesterol medium without PLE are repeatedly subcultured to confirm the results.

2.6 Bacteria Characterization

All isolated strains could be tested for reduction of nitrate, production of indole, hydrolysis of starch, gelatin, esculin, fermentation of amygdalin, arabinose, cellobiose, erythritol, fructose, glucose, glycogen, inositol, lactate, lactose, maltose, mannitol, mannose, melezitose, melibiose, pyruvate, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, threonine, trehalose, and xylose with the standard methods. The final volume of the sterile solutions of substrate to sterile base medium is 2 ml. The test media are inoculated with 0.05 ml of cultures of the isolates grown in base medium. All cultures are incubated at 35°C for 7 days and observed for clotting as an indicator of growth. Fermentation of carbohydrates is determined by a change in pH compared with uninoculated controls and cultures in base medium without carbohydrate. Fermentation of lactate, pyruvate, thronine, reduction of nitrate, production of indole, hydrolysis of starch, gelatin, and esculin are tested by the method of Holdeman (1977).

2.7 Induction of Atherosclerosis in Rabbits and Bacteria Administration

New Zealand white rabbits are fed on a 1% cholesterol enriched diet (Harlan-Sprague Dawley, Inc., Indianapolis, IN, USA) alternating with regular diet every month for a total of 6 months (Ma, 2002). Two weeks after the starting of 1% cholesterol diet, balloon-induced deendothelialization of the aorta is performed using a 4F Fogarty arterial embolectomy catheter (Baxter Healthcare Corporation, Irvine, CA, USA) introduced through the right femoral artery cutdownunderwent under general anesthesia (ketamine 50 mg/kg and xylazine 20 mg/kg, i.m.). The catheter is advanced in a retrograde fashion to the ascending aorta and pulled back three times by 1 ml

balloon volume (Hage-Korban, 1999; Ma, 2003; Ma, 2004a). Atherosclerotic rabbits will be fed with the selected bacteria by adding to the rabbit chow starting at the time of atherosclerosis induction. This has been shown to be effective in previous reports (Gilliland, 2004). Serum cholesterol of atherosclerotic rabbits at six months ranges from 2000 - 4000 mg/dl (Abela, 1995; Ma, 2003).

2.8 Reduction of Serum Cholesterol by Bacteria

The serum cholesterol level in the atherosclerotic rabbits can be measured with a cholesterol diagnostic kit (Sigma Chemical Co., St. Louis, MO, USA). Serum samples will be obtained monthly and for four months then bi-weekly for two months. This can be done via an ear lobe vein stick. This measurement will determine if the bacteria can reduce serum cholesterol of the atherosclerotic rabbits. Cholesterol levels in the bacterial treatment group will be compared to controls (normal rabbits). Rabbits will be observed closely for any potential adverse effects from the administration of bacteria. In these studies, no reports of gastrointestinal symptoms (i.e. diarrhea or vomiting) have been reported.

2.9 Evaluation of Cholesterol Reduction in Cells

Both human aorta smooth muscle and endothelial cells (ATCC, USA) are cultured in a high cholesterol medium (1%). Bacteria with potentially cholesterol-reducing ability will be added to compare the amount of cholesterol in the medium. Two groups of cell cultures are evaluated, one with bacteria that has the cholesterol reducing effects and another with a related strain that does not demonstrate cholesterol reducing effects. These two cultures will be compared with respect to the amount of cholesterol in the cells as well as the amount of cholesterol remaining in the medium.

2.10 Tissue Cholesterol Measurement

To determine the effect of the bacteria on the animal tissue cholesterol concentration, the rabbit (with and without bacteria feeding) liver and arterial cholesterol levels are measured. One cm^2 mid-thoracic and mid-abdominal aortic tissue samples are obtained. Total cholesterol (free and individual ester) in the tissue is measured by high-performance liquid chromatography (HPLC) (Kim, 1984). Each sample of the tissue is ground to a fine powder with anhydrous sodium sulfate and extracted twice with 5 ml of chloroform and methanol mixture (2:1). The extract is dried under nitrogen and re-dissolved in 5 ml of isopropanol. A portion of isopropanol extract is filtered, dried and re-dissolved in the mobile phase. The sample (0.1 ml) is injected into the HPLC column and separated by using a Waters Radial-Pack C18 column eluted isocratically with acetonitrile:isopropanol (45:55 by volume) at 2 ml/min. The absorbance of the eluate is measured at 210 nm with a UV detector. Total cholesterol concentration is calculated by comparing the peak areas of samples with those obtained from the standard (Sigma Chemical Co., St. Louis, MO) (Witztum, 1985).

2.11 Gross Examination

The extent of atherosclerosis is assessed by surface planimetry after sacrifice and exposure of the intimal surface of the aortas. This can be performed on digitized images of the intimal surface obtained by a digital camera. The images can be then downloaded to a computer and the plaque area can be outlined using a custom software package for analysis.

2.12 Light Microscopy

Histology by light microscopy is done to observe the effect of the bacteria on the cholesterol rabbit tissue growth. Arterial and liver tissue specimens are embedded in paraffin, cut and mounted on glass slides. The sections are then stained with hematoxylin and eosin or Masson's trichrome stains. These will be then examined under a light microscope.

2.13 Electron Microscopy

Histology by electron microscopy are done for evaluation of cellular responses. The tissue samples are fixed overnight in 4% glutaraldehyde (Fisher Scientific, Pittsburgh, PA, USA) with 0.1 M phosphate buffer (pH 7.4). Arterial segments (5 mm long) are subjected to critical point drying in liquid CO_2, mounted on stubs and gold-coated in a sputter coater. The intimal surface is examined using a JEOL scanning electron microscope (JEOL Ltd, Model JSM-6400V, Tokyo, Japan). Tissue sections are obtained and processed routinely for ultrastructural examination. Thin sections are stained with uranyl acetate and lead citrate and then examined with a

transmission electron microscope (BEI preamplifier, Au Evirotech Company, Germany).

2.14 Potential Mechanisms of Cholesterol Reduction

In order to detect specific enzymes expressed in the cholesterol-reducing bacteria, which human body cannot produce, it needs to isolate these enzymes. Briefly, selected bacteria are homogenized with physiological buffered solution at 0°C and centrifuged at 10,000 rpm 20 minutes at 0°C. The supernatant is purified by low pressure liquid chromatography or HPLC using gel filtration, ion-exchange and other suitable columns. The solutions at the peaks are collected and the cholesterol-reducing activity is detected in vitro. With these isolates, the specific partials or molecules are characterized and the construction of the molecules is determined. These can be tested in the cell culture set up as a bioassay for cholesterol reduction.

2.15 Rab7 gene regulation

Rab7 may be involved in the process of atherogenesis (Kim, 2002). To identify genes responding to the cholesterol-rich diet and cholesterol-down bacteria treatment, differentially expressed somatic genes are searched from the diet-induced hypercholesterolemic bacteria by differential display reverse transcription-polymerase chain reaction (DDRT-PCR). To visualize the location of elevated Rab7 expression in tissues, patterns of the gene expression are monitored within rabbit aortic tissues by *in situ* hybridization and immunohistochemistry. To find out a potential relationship between the Rab7 and the atherogenesis, the same experiments are conducted with the atherosclerotic plaques obtained from the rabbits.

2.16 Pharmacological Triggering

In this study, the effect of the selected bacteria on the thrombus formation can be also observed. Thrombus is triggered by Russell's viper venom (0.15 mg/kg, i.p., Sigma Chemical Co., St. Louis, MO, USA) and histamine (0.02 mg/kg, i.v., Sigma Chemical Co., St. Louis, MO, USA) given at 48 and 24 hours prior sacrifice (Abela, 1995; Ma, 2003).

2.17 Quantitation of Thrombosis

The total surface area of the aorta, the surface area of aorta covered with atherosclerotic plaque, the surface area of aorta covered with *ante mortem* thrombus are measured to observe the effect of the selected bacteria on the thrombosis. The surface area is measured from images obtained by a color charge-coupled device camera (TM 54, Pulnix, Sunnyvale, CA, USA) and digitized by an IBM PC/AT computer with a color image processing subsystem. The digitized images are calibrated by use of a graticule. Surface area is measured by using of a customized quantitative image analysis package. Also, the number of thrombi on the aortic arch to the distal common iliac branches is counted.

2.18 Glucose and Glucose-6-phosphatase

To observe if glucose metabolism is involved in the bacteria's invading into animal body, rabbit serum glucose concentration and glucose-6-phosphatase activity are detected. Glucose concentration is measured with Sigma Glucose Diagnostic Kit (Sigma Chemical Co., St. Louis, MO, USA). Glucose-6-phosphatase measurement is followed Harper method (Harper, 1965). Briefly, 0.1 ml of tissue homogenate (100 mg tissue/ml) in citrate buffer (0.1 M, pH 6.5) is added into a test tube and incubated at 37°C for 5 minutes. 0.1 ml of glucose-6-phosphate (0.08 M) is added and the sample is incubated at 37°C for 5 minutes, then 5 ml of trichloroacetic acid (10%, w/v) is added and centrifuged at 9,000 rpm at 4°C for 5 minutes. 1 ml of the supernatant is taken into a test tube and 5 ml of ammonium molybdate solution (2 mM) then 1 ml of reducing solution (42 mM 1-amino-2-naphthol-4-sulphonic acid, 560 mM SO_3) is added. The sample is incubated at room temperature for 30 minutes then absorption is measured at 660 nm.

2.19 C-reactive Protein, PAI-1, Interleukin-6 and Tissue Factor Detection

After atherosclerotic rabbits are fed the selected bacteria, inflammation factors C-reactive protein, PAI-1 and interleukin-6 are measured with ELISA method, and tissue factor is measured with immunohistology method (Ma, 2004a; Virk, 2004). This will observe if the bacteria could reduce the inflammation induced by atherosclerotic syndrome of rabbits.

2.20 Heat Shock Proteins Detect

After atherosclerotic rabbits are fed the selected bacteria, heat shock proteins (such as heat shock proteins 25, 60, 70 and 90) are measured by Western Blotting method to observe if the bacteria induce stress response on the rabbits (Ma, 2004).

2.21 Artery Diameter Respond Evaluation

After a period that atherosclerotic rabbits are fed the selected bacteria, the rabbits are sacrificed and both carotid arteries are isolated from each rabbit and placed

in a dual organ chamber then perfused with oxygenated physiologic buffered solution (PBS) (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 2 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 22.6 mM, glucose 11.1 mM and Na_2EDTA 0.03 mM) at 60 mmHg and 2.5 ml/minute flow rate at 37°C. Baseline vasodilation is determined using norepinephrine (NE, 1×10^{-6} M) preconstriction and pharmacological challenge is then performed with acetylcholine (Ach, 1×10^{-5} M) and sodium nitroprusside (SN, 1×10^{-5} M) successively. Vessel diameter is measured by a computer planimetry system (Figure 1). The data are calculated according to the formulas: Ach-NE (%)=(Ach-NE)/NE×100 and SN-NE (%)=(SN-NE)/NE×100 separately, where Ach, NE and SN represented the diameter (mm) of the arteries that will be perfused by the PBS containing a corresponding chemical (Figure 1).

2.22 Statistical Analysis

SigmaStat (Sigma Chemical Co., St. Louis, MO, USA) or Microsoft Excel software is used for data statistical analysis. $P<0.05$ is considered statistically significant difference. Measured data are reported as mean±SD or mean±SE. The student t-test is used for comparison.

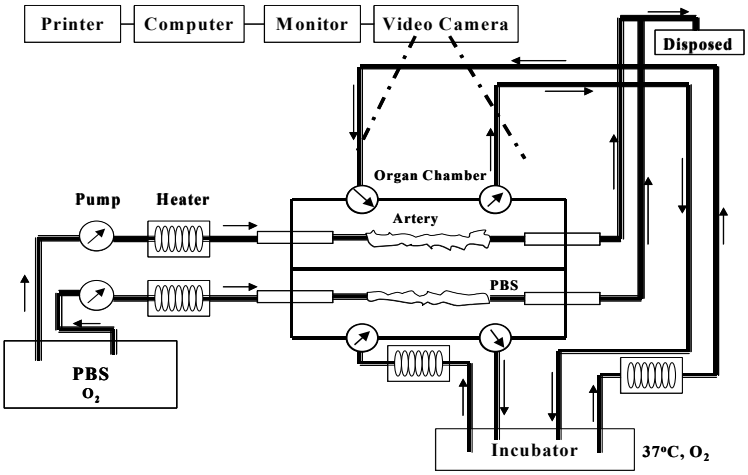

Figure 1. Dual organ chamber with separate perfusion using oxygenated physiological buffered solution at 37°C. Vessel diameter is measured by a computer planimetry system.

2.23 Potential Difficulties and Limitations and the Alternative Approaches to Achieve the Aims

The selection of the bacteria stains is difficult and it is labor intensive. However, bacteria could play role in cardiovascular diseases (Kuramitsu, 2001). There is a potnetial that the rabbits may not tolerate the oral bacteria. This could be addressed by using lower amounts of bacteria given or to treat more intermittently. Veterinary physicians could be consulted to help address these issues.

3. Results and Discussions

Bacteria from dog intestine were cultured in the medium with different cholesterol content (0, 50, 100, 200, 300, 400, 500, 600, 800, 1000 mg/dl). We noted the growth of bacterial colonies in culture that was inversely related to cholesterol concentration (i.e. fewer colonies in high cholesterol concentration media).

The purpose of this article is to offer the idea and techniques of selecting bacteria from carnivores that will reduce cholesterol level in human. There are many reports to show that bacteria in animal host decrease blood cholesterol levels by either decreased total intestinal absorption and removal through solid excretion, or by interrupting the entero-hepatic cycle of bile acids, such as lactobacillus bacteria and bifidobacteria (James, 1999). As our study results, we selected bacteria from dog gut that decrease cholesterol level in the human cell culture dish. As this article is to describe the possibility and technique to isolate and characterize bacteria from carnivores that inducing cholesterol level in animal blood, it will not give the detail of bacteria selection results here. We hope that the information in this article could help readers in do further studies in the cardiologic medicine that will be benefit the medical research and health improvements.

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Patient Referral at the Grass-roots Level in Pakistan

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Abstract: Patient referral services constitute an integral part of any well functioning health system. In the public health sector in Pakistan, the first rung in the referral hierarchy is the Lady Health Worker (LHW) of the National Programme for Family Planning and Primary Health Care. Most independent evaluations conducted to date have given satisfactory reports of LHWs performance despite their limitations, and acknowledged the importance of their role in first level referral. However, the outcomes and consequences of referral made by the LHWs are largely unknown. One outcome, patient dissatisfaction, has been widely recognized as a measure of ascertaining how well health services are being delivered. It is imperative to learn about the referral outcomes to ascertain continuity of care, cost effectiveness of management and ensuring the appropriate case mix at the respective health facilities. Patients referred were interviewed in a cross-sectional study conducted in Karachi, Pakistan. Structured questionnaire was used to collect the data on demographic attributes, outcome of referral and satisfaction with regard to the services at the referral health facilities. About fifty six percent of patients were referred for the management of various medical and/or surgical conditions. The median cost of the treatment was 200 PKR and 1230 PKR as outpatient and in-patient respectively. About 24% of the patients reported to be cured. With regard to patients' satisfaction, 31.6% of patients were not satisfied with their management at the referral facilities. Final multiple logistic regression analysis showed that long distance to health facility (AOR, 3.54; CI: 1.36-9.19), long time to reach the referral facility (AOR, 3.72; CI: 1.06-13.04), borrowing of money for treatment (AOR, 2.14; CI: 1.18-3.89) and outcome of condition (AOR, 9.08; CI: 3.33-24.67) were significantly associated with patient dissatisfaction. LHWs are the first contact for many people with the health system in general and with the referral system in particular. However, after being referred by the LHWs, patients are left more or less unsupported by the formal system, causing in many cases, an inevitable waste of resources, unnecessary and avoidable morbidity, and social and mental stress. [Nature and Science. 2004;2(4):18-27].

Key Words: lady health workers; referral system; patient dissatisfaction; Pakistan

1. Introduction

The modern referral system was first initiated in the United Kingdom and was well established by the 1948 nationalization of hospitals. Patient referral services constitute an integral part of any well functioning health system. The goal of referral services is to ensure that patient is dealt with at the appropriate level health facility, and receive cost effective and quality management (Mariker, 1998). In addition, referral also serves to provide linkages between primary and secondary and tertiary care.

The concept of a health care hierarchy of a referral system is based on the systems notion, in which certain population within a given catchment area is provided specified levels of care. With the realization of the importance of primary health care (PHC), the skill pyramid of the conventional health care hierarchy with the community health workers (CHWs) at the bottom and the physicians at the top has been tipped to the side (Mariker, 1998; Stefanini, 1999) with patients not only moving to higher levels (secondary or tertiary care facilities) but also to lower levels according to individual needs. Such a dynamic multi-level flow of patients between varying cadres of health care providers is seemingly complicated to manage but essential to quality and cost effectiveness. Unfortunately, in many developing countries people tend to by pass the first level care

facility, which results in overburdening of higher-level facilities (Kordy, 1991).

In the public health sector in Pakistan, the first rung in the referral hierarchy is the Lady Health Worker (LHW) of the National Programme for Family Planning and Primary Health Care, which was initiated in 1999. Apart from the various other PHC activities that LHWs undertake, they also refer patients and guide individuals from the grass-roots to the appropriate health facilities (Siddiqi, 2001). No study has been identified by the authors that have studied referral rates by CHWs/LHWs. However, referral rates by the primary care physicians to specialists/consultants in well-structured health systems range from 2% to 28% (Siddiqi, 2001; Ministry of Health, 1993; Sindh Survey Report, 2002). One may assume that referral rates by community health workers would be higher than the rates by GPs. A local study conducted in Karachi, Pakistan, has reported a referral rate of 55% by LHWs, which seems to be fairly high. Some of the reasons suggested by the authors included the limitations of the LHW to manage more than very basic medical conditions and lack of resources like drugs and other medical supplies (Alper, 1994).

Independent evaluations conducted to date have given satisfactory reports of LHWs performance despite the limitations, and acknowledge the importance of their role in first level referral (Sindh Survey Report, 2002). However, the outcomes and consequences of referral made by the LHWs are largely unknown. It is imperative to learn about the referral outcomes to be able to make certain continuity of care and hence quality of care, curtailing costs and ensuring the appropriate case mix at the respective health facilities (Alper, 1994; Counter, 1989).

In Pakistan, an estimated 70 to 80% of the population uses private services for their health needs, the public sector, if regulated in a structured manner, is a very important source of equity in health care (Counter, 1989) especially with regard to PHC (where the private sector has a limited role). On the other hand, reports show that LHWs cater to a significant proportion of the population in areas they serve and refer to both government and private facilities. Thus LHWs are an important link in 'private-public partnership', where both the sectors may complement each other in providing equitable and quality health services (Sindh Survey Report, 2002). Unfortunately, in most of the developing countries, including Pakistan, the equation is tipped heavily towards the private sector and public sector utilization is dismally low (Afsar, 2003). One important reason cited for this low utilization is client dissatisfaction. Patient satisfaction in health care has been widely recognized as a measure of ascertaining how well health services are being delivered as well as accessed and utilized (Maynard, 2001). The subsequent role of the LHW within the whole system of health service delivery and its consequences on the patient need to be viewed in a holistic manner. The study presented here is part of a larger study that looked at the proportion of referrals by the LHWs and factors affecting unsuccessful referral (patients referred by the LHWs who did not visit referral site within 30 days). This study aims 1) to assess the medical and financial consequences of successful referrals (patients who were referred and visited the referral site within 30 days) to the higher-level health facilities by the LHWs and 2) to identify the factors associated with patients' dissatisfaction, attending the referral health facilities in Karachi, Pakistan. The study protocol was approved by the institutional ethics committee board of The Aga Khan University.

2. Methodology

2.1 Design and setting

This cross-sectional study was conducted from November 2000 to March 2001 in the District West of Karachi. Karachi is the largest city of Pakistan with a population of 14 million. Karachi's economic dominance has led to the massive influx of people from all areas of the country making it a multi-linguistic, multi-ethnic and multi-cultural region, and has been described as 'mini Pakistan'. For administrative purpose, Karachi is divided into five districts: East, South, West, North and Malir. According to 1998 census report the total population of district West is 2105923 with 1149200 (54.6%) males and 956723 (45.4%) females (Khan, 1996). About 900 LHWs are currently providing primary health coverage in Karachi, among them 337 LHWs were based at 8 BHUs in District West, who report to the District Health Office (DHO), District West (currently Executive District Officer Health).

2.2 Recruitment of subjects

The list of LHWs providing services in District West was obtained from the District Health Officer West. LHWs were randomly selected through a random number table. Records of the last one-month (preceding the study period) of selected LHWs were reviewed; the latest eight patients referred to various health care facilities from the visited houses were selected. The study team visited and interviewed the selected individuals. Sub-set of the patients visited, who were referred to various level health facilities by the LHWs and who later attended their respective referral facilities were included in this part of the study (Figure 1).

2.3 Data collection

After obtaining informed consent, a pre-tested structured questionnaire was administered through trained staff. Parents or immediate family members who were interviewed in case subject were <15 years of age. Data were collected regarding demographic characteristics (age, sex, monthly income, employment status), process of referral (distance of the referral facility from residence, means of transportation used, visited the facility alone/with someone, cost of treatment) and patient satisfaction (with regard to services and management at the referral sites, clinical services available).

2.4 Data management and analysis

Data were analyzed according to the two objectives of the study. To observe the outcome of patient referral, descriptive statistics was carried out: mean, standard deviation and median were calculated for continuous variables, and percentages for the categorical variables. To identify factors associated with patient dissatisfaction, association between outcome variable (satisfaction versus dissatisfaction) and independent variables (patients' socio-demographic characteristics and hypothesized factors for patient dissatisfaction) were sought. Exposure variables were categorized into two or more levels, assuming as reference the category deemed to have the lowest risk of dissatisfaction. Subgroups were analyzed by using Chi-square and Fisher Exact Test.

To observe the individual effects of each exposure variable, potential mutual confounders were simultaneously controlled by means of stepwise multiple logistic regression analysis and odds ratios (ORs) with their 95% confidence intervals (CIs) were computed. After arriving at main effects model, plausible interaction terms were evaluated. The goodness-of-fit of the final multivariate model was checked by Pearson Chi-square test**.** An epi-info *(version 6.04. Atlanta, GA; Center for Disease Control and Prevention; 1995)* based programme was developed to enter the data, and Statistical Package for Social Sciences *(version 8.0. Chicago; IL: SPSS Inc; 1996)* was used for analysis.

3. Results

Of the 247 patients who attended the referral site, 155 (62.8%) were females and 92 (37.2%) were males. The mean age of the patients was 37.6±21.2 SD, and majority 140 (56.7%) of them were of age >35 years. About 60% of the patients were married. More than half 126 (51.0%) of study subjects had no formal schooling. On the basis of mother tongue, we categorized the ethnicity into four groups, majority 140 (56.7%) of them were Mohajirs. Only 54 (21.9%) patients had reimbursement/insurance facility for their medical bills. Table 1 describes the socio-demographic characteristics of the referred patients.

3.1 Referral outcome

Among patients who attended the health facility, 140 (56.6%) were referred for the management of various medical/surgical and gynecological/obstetrical conditions, 61 (24.7%) for investigations, 35 (14.2%) for diagnosis and 11 (4.5%) on patient request. Only 4% of patients visited government facilities, the rest having visited private physicians (61.5%) and in-formal practitioners 63 (25.5%). One hundred seventeen (47.4%) patients were prescribed 0-3 drugs, 78 (31.6%) 4 to 6 drugs and 52 (21.1%) more than 6 drugs. One hundred and seventy-seven patients (71.7%) were treated as out-patients, while 70 (28.3%) were treated as in-patients. The median cost of the treatment was 200 PKR and 1230 PKR as out-patient and in-patient respectively. One hundred and two (41.3%) patients had borrowed money to finance their treatment. Due to the current episode of ill health, 59 (23.7%) of patients stayed away from their work for 2-8 days. About 25% of patients reported improvement in their conditions with the management they received at the referral facilities.

3.2 Factors associated with patient dissatisfaction

Seventy-eight (31.6%) patients were not satisfied with the management they received from the referral health facilities. When asked about reasons for dissatisfaction, 28 (11.3%) cited that they did not get better, 18 (7.3%) said that the treatment was too expensive, 12 (4.9%) complained about the long waiting time, 12 (4.9%) said that they were not given any medications and 8 (3.2%) complained the rudeness of the health facility staff.

We found no difference in the socioeconomic and demographic profile of satisfied and dissatisfied patients groups ($p>0.05$). Univariate analysis for the factors associated with dissatisfaction in patients who attended the referral health facilities is given in Table 2. Final multiple logistic regression analysis (Table 3) revealed that long distance to referral facility (>3 km *vs* <1 km, Adjusted OR, 3.54; CI: 1.36-9.19), long time to reach the referral facility (>60 minutes *vs* <30 minutes, Adjusted OR, 3.72; CI: 1.06-13.04), borrowing of money for treatment (Yes *vs* No, Adjusted OR, 2.14; CI: 1.18-3.89) and outcome of condition (never got better *vs* cured, Adjusted OR, 9.08; CI: 3.33-24.67) were significantly associated with referral patient dissatisfaction. The final multivariate model fitted well as confirmed by the Pearson goodness-of-fit test ($p=0.813$).

4. Discussion

The present study set out to assess the outcome of patient referral and to identify the factors associated with dissatisfaction in patients attending the referral health facilities. We found that the major clienteles of LHWs were women and children. Considering that women are often restricted to visit a health facility by themselves in most of the socio-cultural settings in Pakistan, LHWs are an important source of PHC at the grassroots-level. Other important groups of patients being catered to by the LHWs were adolescents (11 to 19 years) and the elderly (>60 years), both groups of which often have less access to health services.

4.1 Referral outcome

The majority of patients were referred for the management of various medical and/or surgical conditions, which is consistent with other research work (Barker, 1991; Majumdar 1997; DCR, 1998). Among total patients referred substantial number of referrals (28.3%) were consequently admitted as in-patients. This proportion may be significantly reduced if LHWs were better trained and supplied to manage acute conditions and provide first aid.

One may argue that the less number of referred patients visiting government facilities reflects failure of the LHWs programme to increase government facilities usage. However, without the necessary improvements in quality of these health facilities, LHWs can do little to improve utilization. On the contrary, LHWs may face embarrassment and loss of credibility when the patients they have referred return disappointed and dissatisfied from these health facilities (Nordberg, 1996).

The median cost incurred by the patients attended as out-patients was 200 PKR, constituting 6% and 3% of the monthly income of the lowest and the next higher income groups respectively, a large proportion of the monthly wage to spend on a single episode of illness. On admission as an in-patient, the total treatment cost rose to six times i-e 1230 PKR. The immense economic burden can be judged by the fact that a large number (41.3%) of people had to borrow money in order to finance their treatment. There was also appreciable loss of productive workdays. Hence, the monetary consequences of referral are significant enough to emphasize on curtailing unnecessary referrals.

With current emphasis on identification and symptomatic treatment, a visit to a health care provider inevitably results in prescription of multiple drugs even if they may not be medically justified (Harold, 1996; Halm, 1997). In our study, almost one third (31.6%) of patients were prescribed 4 to 6 drugs and about one fifth (21.1%) more than 6 drugs. The outcomes of such 'apparent' injudicious prescription of drugs results in increasing appearance of resistant strains of infections, undesired side-effects, lack of patient compliance and a waste of valuable resources (Sturm, 1997; Das, 2001). Ironically, one reason for dissatisfaction with management was that patients were not given any medications. This typifies the cultural paradigm of the communities' expectations of a health care provider in which some medication given, usually in the form of drugs or injections, is perceived as a sign of quality (Afsar, 2002). The physicians may be partially responsible for this induced demand for drugs. The LHW in her role as a

health educator can help create awareness in this regard. LHWs contacted majority (92.7%) of their referred patients afterwards to enquire about outcome of their referrals. This practice is in line with the process of feedback and continuity of care, and augments well for the LHW-client relationship.

4.2 Factors associated with patient dissatisfaction

One third of the patients reported to be dissatisfied with the management at the referral sites, which is similar to studies done in other settings (Das, 2001; Millar, 2001). Final model showed that long distance and long travel time to health facility were significantly associated with patient dissatisfaction. In Pakistan, despite an extensive network of primary health facilities, quite often individuals with ailments that could easily be managed at lower level tend to bypass and visit tertiary care institutions and thus over-burden the outpatient departments of major hospitals (Sturm, 1997; Siddiqi, 2001). Inevitably, these patients spend more time to travel longer distances and have to wait longer to be seen by the physician. In developing countries including Pakistan, the effect of distance on service utilization becomes stronger, when combined with the dearth of transportation and poor roads, as a cause of dissatisfaction (Coulter, 1995; Khan, 2000; Kersnik, 2001). The importance of a well-established transport mechanism has been cited as an essential part of a referral system (Bhuiya, 1995). Even though lack of resources may limit the possibility of developing and maintaining such a mechanism, the author has come across a Community Based Organization (CBO) in a remote desert area (Therdeep) in Pakistan that has developed a system whereby the community contributes towards a pool of money reserved for transportation costs for patients in need of serious medical attention. Local health authorities may replicate such novel initiatives wherever possible.

Our study showed that people spend a large proportion of their income on health care, even if they have to borrow money. In our study, borrowing of money for treatment was found associated with patient dissatisfaction. This may be partly explained by the fact that borrowing of money not only increases the financial burden but also compromises self-esteem. One could hypothesize that these people then, would avoid visiting the facilities at the start of their illness and delay seeking care until complications force them to do so. This inevitably raises treatment cost further while at the same time increasing the morbidity and mortality (Janse, 1999).

Our data showed that patients who 'did not get better' were more likely to be dissatisfied compared to patients who reported to be cured with the management they received. This is understandable; though we are not in a position to comment on why their conditions did not improve. In our study 3.2% patients reported 'rudeness of the health facility staff', a reason for dissatisfaction. Staff attitude has been reported as an important factor for patient satisfaction (Katung, 2001). It is hypothesized that the success of informal sector (unregistered practitioners and quacks) in attracting patients may be attributed to their good interpersonal skills and client-provider relationship (Campbell, 1997; Aljunid, 1996; Sharaf, 2003). Other important factors for patient dissatisfaction such as absence of insurance, repeated visits to referral site and visiting referral facility alone were not found significant in the multivariate analysis.

Our study does have some important limitations. Firstly, we could not assess the appropriateness of the referrals made by the LHWs. Secondly, due to limited scope of the design and the large number of different referral sites clients were referred to, we did not study the provider-client interaction at referral health facilities which we believe could have strengthened our discussion with regards to patient dissatisfaction.

5. Conclusions

The LHWs are providing invaluable services with regards to PHC and support to patients. Our current analysis and earlier work suggest that despite an inevitable large referral rate, the LHWs are effectively functioning as the first contact of many people with health system in general and specifically with the referral process. Beyond this, there is currently little data available to argue against our hypothesis that the patient after being referred by the LHWs from the grass root level is left unguided and not sufficiently supported by the heath system, causing in many cases, an inevitable waste of resources, unnecessary and avoidable morbidity and social and mental stress.

More research is needed to follow patients as they interact with various levels in the health system so that loopholes causing wastage and delays are minimized and the health system be structured to provide efficient, effective and equitable health services to all.

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Table 1. Characteristics of the patients (referred by the Lady Health Workers) who attended the referral health care facilities in Karachi, Pakistan (n=247).

	#	%
Age (completed years)		
<15	35	14.2
15-35	72	29.1
> 35	140	56.7
Gender		
Male	92	37.2
Female	155	62.8
Ethnicity		
Mohajir	140	56.7
Sindhi	21	8.5
Pathan	48	19.4
Punjabi	21	8.5
Baloch and others	17	6.9
Education of the donor		
Illiterate	126	51.0
Primary to Secondary	96	27.9
Intermediate and above	52	21.1
Marital status		
Single	24	9.7
Married	148	59.9
Divorced/widowed/separated	32	13.0
Not applicable (age less than 15 years)	43	17.4
Household income (PKR/month)*		
≥ 6000	67	27.1
3000-<6000	120	48.6
< 3000	32	13.0
Not applicable (age<15 years)	28	11.3
Number of household		
≤5	73	29.6
6-10	137	55.5
>10	37	15.0
Beneficiary status (Insurance)		
Yes	54	21.9
No	193	78.1

* Pakistani Rupee (1 $= 58 PKR)

Table 2. Univariate analysis of the factors associated with patient dissatisfaction at the referral health facilities in Karachi, Pakistan.

Associated factors	Dissatisfied Patients		Satisfied Patients		OR†	95% CI‡
Age (years)						
> 35	43	(55.1)	97	(57.4)	1.00	-
15-35	24	(30.8)	48	(28.4)	1.03	0.61-2.07
<15	11	(14.1)	24	(14.2)	1.13	0.47-2.29
Gender						
Male	26	(33.3)	66	(39.1)	1.00	-
Female	52	(66.7)	103	(60.9)	1.28	0.73-2.25
Ethnicity						
Mohajir	53	(67.9)	87	(51.5)	1.00	-
Punjabi	2	(2.6)	19	(11.2)	1.49	0.59-3.75
Pathan	10	(12.8)	38	(22.5)	0.43	0.19-0.94
Sindhi	10	(12.8)	11	(6.5)	0.17	0.04-0.77
Baloch and others	3	(3.8)	14	(8.3)	0.35	0.09-1.28
Education						
Intermediate & above	13	(17.3)	39	(24.2)	1.00	-
Primary - secondary	24	(32.0)	45	(28.0)	1.61	0.72-3.56
Illiterate	38	(50.7)	77	(47.8)	1.45	0.69-3.0
Marital status						
Single	22	(28.2)	45	(26.6)	1.00	-
Married	47	(60.3)	101	(59.8)	0.95	0.51-1.76
Separated\Divorced \Widowed	9	(11.5)	23	(13.6)	0.80	0.32-2.02
Beneficiary Status						
Yes	18	(23.1)	36	(21.3)	1.00	-
No	60	(76.9)	133	(78.7)	0.90	0.47-1.72
Monthly Income**						
≥ 6000	6	(9.0)	26	(17.1)	1.00	-
3000 - <6000	43	(64.2)	77	(50.7)	2.42	0.92-6.34
<3000	18	(26.9)	49	(32.2)	1.59	0.56-4.49
Ever been referred before						
Yes	40	(56.4)	85	(50.3)	1.00	-
No	34	(43.6)	84	(49.7)	0.88	0.49-1.57
Attended the referral site						
Same day	19	(24.4)	77	(45.6)	1.00	-
Within a week	44	(56.4)	64	(37.9)	2.79	1.48-5.24
After one week	15	(19.2)	28	(16.6)	2.17	0.97-4.84
Someone accompanied						
Yes	60	(76.9)	145	(85.8)	1.00	-
No	18	(23.1)	24	(14.2)	1.81	0.92-3.58
Went to referral site by						
Walk	24	(30.8)	64	(37.9)	1.00	-
Transport	54	(69.2)	105	(62.1)	1.37	0.78-2.43

Continue Table 1:

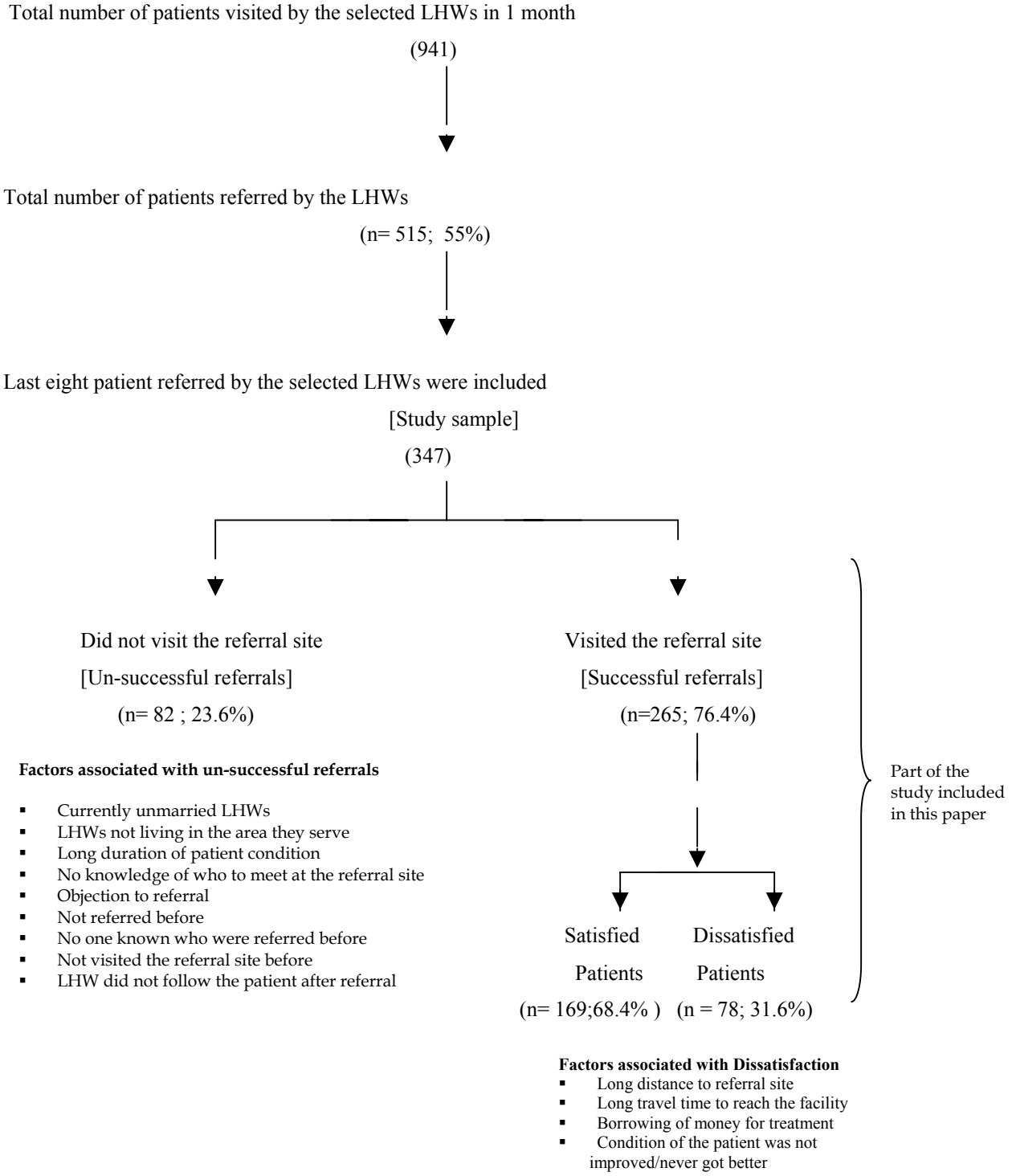
Time to reach the referral site (minutes)						
<30	30	(38.5)	88	(52.1)	1.00	-
30 - 60.	38	(48.7)	75	(44.4)	1.49	0.84-2.62
>60	10	(12.8)	6	(3.6)	4.89	1.64-14.59
Number of visits to the referral site						
0 - 2						
3 – 5	26	(33.3)	51	(30.2)	1.00	-
> 5	32	(41.0)	66	(39.1)	0.95	0.51-1.79
	20	(25.6)	52	(30.8)	0.75	0.38-1.52
Total cost (Rupees)						
<100	20	(25.6)	38	(22.5)	1.00	-
100-<500	24	(30.8)	51	(30.2)	0.89	0.43-1.85
500-2000	20	(25.6)	44	(26.0)	0.86	0.41-1.84
>2000	14	(17.9)	36	(21.3)	0.74	0.33-1.68
Borrowed money						
No	36	(46.2)	109	(64.5)	1.00	-
Yes	42	(53.8)	60	(35.5)	2.12	1.23-3.66
Admitted as inpatient						
No	55	(70.5)	122	(72.2)	1.00	-
Yes	23	(29.5)	47	(27.8)	1.08	0.60-1.96
Stayed away from work/school (days)						
0-2	23	(30.7)	44	(27.3)	1.00	-
3-8	11	(14.7)	45	(28.0)	0.45	0.19-1.02
>8	41	(54.7)	72	(44.7)	1.13	0.61-2.12
Outcome of condition						
Cured	9	(11.5)	52	(30.8)	1.00	-
Under consultation	44	(56.4)	98	(58.0)	2.59	1.18-5.72
Never got better	25	(32.1)	19	(11.2)	7.59	3.01-19.17
Distance from the referral site (Kilometer)						
< 1	10	(12.8)	48	(28.4)	1.00	-
1-3	31	(39.7)	65	(38.5)	2.29	1.02-5.12
> 3	37	(47.4)	56	(33.1)	3.17	1.43-7.04

- **Statistically significant ** 28 refused to answer the question † Confidence interval ‡Odds ratio**

Table 3. Multivariate regression analysis of the factors associated with patient dissatisfaction, who attended the referral health facilities in Karachi, Pakistan.

Associated factors	**Adjusted OR†**	**95 % CI‡**
Distance from the referral site (kilometer)		
< 1	1.00	-
1-3	2.23	0.93-5.34
> 3	3.54	1.36-9.19
Time to reach the referral site (minutes)		
<30	1.00	-
30 - 60.	0.85	0.41-1.73
>60	3.72	1.06-13.04
Borrowed money		
No	1.00	-
Yes	2.14	1.18-3.89
Outcome of condition		
Cured	1.00	-
Under consultation	2.58	1.10-6.06
Never got better	9.08	3.33-24.67

† Confidence interval ‡ Odds ratio



***Data of 18 patients were incomplete and not included in the analysis**

Figure 1. Schematic description of enrollment of study subjects

Preparation and Structure of a Novel Crystalline Adduct of Two Organotin Compounds [Ph_2Sn-$SnPh_2$• (μ-$PhCO_2)_2$] and (Ph_3Sn-$SnPh_3$)

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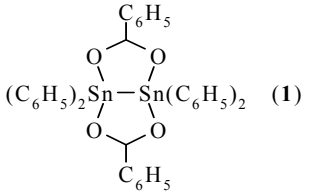
Abstract: A new interesting crystalline adduct was prepared by the radical reaction of triphenyltin hydride and benzoyl peroxide. The crystalline adduct structure was determined. It is found out that the crystalline adduct is formed by two organotin compounds [**1**: Ph_2Sn-$SnPh_2$• (μ-$PhCOO)_2$, **2**: Ph_3Sn-$SnPh_3$, 1 / 1] which are interacted by intermolecular Van der Walls forces. The Ph_4Sn_2 units in compound **1** are bridged by two isobidentate benzoates resulting in five-coordinated tin atoms. The Sn-Sn bonds are found in organotin compound **1** and **2** and the Sn-Sn bond lengths in **1** and **2** are 2.6983(10) and 2.8040(11) Å respectively. A possible mechanism of the formation of the crystalline adduct is also suggested. [Nature and Science. 2004;2(4):28-33].

Keywords: organotin compounds; crystalline adduct; preparation; Sn-Sn bond; crystal structure

1. Introduction

Since the synthesis of organotin compounds was first reported by Frankland in 1849, the syntheses and application of organotin compounds have attracted chemists' considerable attention (Pan, 1990; Gielen, 1992; Crowe, 1980; Gielen, 1996). Hutton et al developed a novel route to the preparation of *β*-alkoxycarbonylethyltin trichlorides, $ROCOCH_2CH_2SnCl_3$ (1978). The research results show that these compounds have a variety of coordination geometries regarding the tin atom. We have reported the synthesis, structure and transesterification of the derivatives of 2-alkoxycarbonylethyltin trichlorides (Tian, 1998; 1998; 2000). With an attempt to get the derivatives of 2-alkoxycarbonylethyltriphenyltin ($Ph_3SnCH_2CH_2CO_2R$) by the radical reaction of triphenyltin hydride, acrylate and benzoyl peroxide, we got a crystalline adduct of organotin compound **1** and **2** (the structures are shown as follows) instead. In this present paper, this novel mixed single crystal structure is reported, and a possible mechanism for the formation of the crystalline adduct is suggested.



(**1**)

$(C_6H_5)_3Sn-Sn(C_6H_5)_3$ (**2**)

2. Experimental

2.1. Materials and physical measurements

All chemicals were of reagent grade or analytical grade, and used without further purification. The solvents used were dried over $CaCl_2$ and distilled before use. Carbon and hydrogen analyses were carried out with a Perkin-Elmer 2400II elemental analyzer, and tin was determined gravimetrically as SnO_2. The results of elemental analyses are in agreement with the theoretical calculations (± 0.5 %). IR spectra were recorded on a Nicolet 470 FT-IR spectrophotometer in KBr pellet. ^1H NMR spectra were carried out on a DPX-400 FT NMR spectrometer. Chemical shifts δ are given in ppm relative to that of chloroform (δ = 7.24 ppm). The structure was determined on an R-AXIS-IV diffratometer with graphite monochromated Mo-Kα radiation.

2.2. Preparation of triphenyltin hydride (Ph_3SnH) (Kuivila, 1961)

A solution of triphenyltin chloride (Ph_3SnCl, 38.5 g, 0.1 mol) dissolved in ether (150 cm^3) was placed in a three-necked, Ar-flashed flask. $LiAlH_4$ (1.56 g, 0.041 mol) was added slowly under 5°C. The reaction mixture was stirred vigorously at 5°C for 15 minutes and placed at room temperature for 3 hours. Then, 100 cm^3 of cool water was added dropwise. The ether layer was separated and the water layer was extracted with ether (2×50 cm^3). The mixed extract was washed with water (2×50 cm^3), dried with anhydrous sodium sulphate, and then evaporated to dryness to afford a crude oily product. The pure triphenyltin hydride (28 g, 79.8%) was obtained by reduced pressure distillation (162-168°C / 0.5 mmHg) as an oil.

2.3. Preparation of the crystalline adduct {[Ph_2Sn-$SnPh_2$• (μ-$PhCOO)_2$] and (Ph_3Sn-$SnPh_3$)}

Triphenyltin hydride (0.005 mol) and benzoyl peroxide (0.005 mol) were dissolved in ether (100 cm^3). The solution was stirred at 35 °C for 30 hours. The crude solid product was obtained after the removal of the solvent. The pure crystalline adduct was obtained by the recrystallization from chloroform, yield 6.4 g of the crystalline adduct. *Anal*. Found: C, 59.66; H, 4.19, Sn, 31.58 %. Calc. for $C_{74}H_{60}O_4Sn_4$: C, 59.72; H, 4.06, Sn, 31.91 %. IR: 3037(C-H); 1600, 1587 (phenyl ring); 1528, 1410 (COO). ^1H NMR ($CDCl_3$): δ = 6.78~7.46 (m, 30H, ArH).

2.4. Crystal structure determination of the crystalline adduct

A summary of the fundamental Crystal data for the crystalline adduct is given in Table 1. A colorless crystal of the crystalline adduct with the dimensions 0.30×0.20×0.20 mm was mounted on a R-AXIS-IV diffratometer with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The cell parameters were refined by least-squares fit of all reflections collected. The structure was solved by heavy-atom method. All refinements were performed by full-matrix least-squares on F^2. The positions of the hydrogen atoms were calculated assuming ideal geometries and refined with isotropic thermal parameters. All non-hydrogen atoms were refined with anisotropic thermal parameters.

3. Results and Discussion

3.1. Preparation of the crystalline adduct

With an attempt to get the derivatives of 2-alkoxycarbonylethyltriphenyltin ($Ph_3SnCH_2CH_2CO_2R$) by the radical reaction of triphenyltin hydride, acrylate and benzoyl peroxide, a kind of white crystal was obtained. The characterization by EA, IR, ^1H NMR showed that the product was a mixture of organotin compound **1** and **2** with the ratio of 1:1. The mixture was also obtained by the radical reaction of triphenyltin hydride and benzoyl peroxide in ether. The crystalline adduct used for the structure determination was obtained by the recrystallization from chloroform.

3.2. Crystal structure of the novel crystalline adduct

The crystal structures of the crystalline adduct**,** compound **1** and **2** are shown in Figure 1, Figure 2 and Figure 3. The crystal packing of the crystalline adduct is shown in Figure 4. Bond lengths and bond angles are given in Table 2 and Table 3. It is shown that the organotin compound **1** and **2** are interacted by intermolecular Van der Walls forces. The Ph_2Sn-$SnPh_2$ units in compound **1** are bridged by two isobidentate benzoates resulting in five-coordinated tin atoms. The bond lengths (in the compound **1**) of Sn(1)-Sn(1A), Sn(1)-O(1), Sn(1)-O(2), Sn(1)-C(6) and Sn(1)-C(19) are 2.6983(10), 2.274(4), 2.312(3), 2.143(5) and 2.157(5) Å respectively, the bond angles of C(6)-Sn(1)-C(19), C(6)-Sn(1)-Sn(1A), O(1)-Sn(1)-Sn(1A), O(1)-Sn(1)-C(6) and O(1)-Sn(1)-O(2) are 115.3(2), 123.39(15), 84.88(10), 93.1(2) and 169.02(13)°, respectively. The bond lengths (in compound **2**) of Sn(2)-Sn(2A) and Sn(2)-C(31) are 2.8040(11) and 2.159 Å, the bond angles of C(31)-Sn(2)-C(37) and C(31)-Sn(2)-Sn(2A) are 106.9(2) and 111.5(2)°. The Sn-Sn bond lengths of the organotin compound **1** and **2** are shorter than the distance between tin atom in the metal crystal (2.82 Å). These results demonstrate that Sn-Sn bond is formed, which is a very valuable result.

3.3. Mechanism for the formation of the crystalline adduct

The formation of the crystalline adduct is very

interesting. So, the formation mechanism for the crystalline adduct is studied. The formation reaction of the crystalline adduct undergoes a radical mechanism, and a possible mechanism of the formation is suggested (Figure 1). The homolysis of benzoyl peroxide gives two benzoyloxy radicals, which abstract two hydron atoms from two triphenyltin hydride, producing two triphenyltin radicals and two molecules of benzoic acid. And then, these two triphenyltin radicals combine to form hexaphenyl distannane (**2**). Finally, the substitution of two phenyl groups in hexaphenyl distannane by two benzoate anions, producing tetraphenyl distannane dibenzoate complex (**1**).

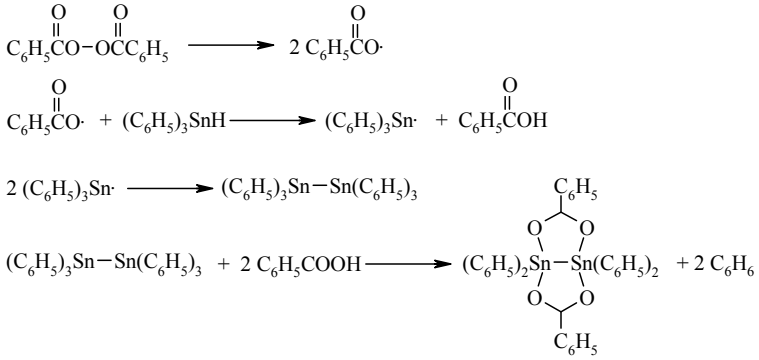


Figure 1. A possible mechanism for the formation of the crystalline adduct

Table 1. Crystal data for the crystalline adduct {[Ph_2Sn-$SnPh_2$• (μ-$PhCOO)_2$] & (Ph_3Sn-$SnPh_3$)}

Empirical formula	$C_{74}H_{60}O_4Sn_4$
Formula weight	1487.98
Temperature (K)	291(2)
Crystal system	triclinic system
Space group	P-1
Unit cell dimensions	
a (Å)	9.242(2)
b (Å)	12.248(2)
c (Å)	14.511(3)
α (°)	95.73(3)
β (°)	95.07(3)
γ (°)	94.71(3)
V (Å^3)	1621.2(6)
Dc (Mg mm^{-3})	1.524
Z	1
F(000)	736
Absorption coefficient *μ* (mm^{-1})	1.570
λ (Å)	0.71073
Crystal size (mm)	0.30 × 0.20 × 0.20
Theta range for data collection (°)	1.42 ≤ θ ≤ 27.58
Index ranges	-12 ≤ h ≤ 11, 0 ≤ k ≤ 15, -16 ≤ l ≤ 16
Reflections collected	5343
Independent reflections	5343
R_{int}	0.0000
Refinement method	full-matrix least-squares on F^2
Data / restraints / parameters	5343 / 0 / 371
Goodness-of-fit on F^2	1.114
final R indices [$I>2\sigma(I)$]	R_1 = 0.0712[a], wR_2 = 0.2050 [b]
R indices (all data)	R_1 = 0.0850 [a], wR_2 = 0.2258 [b]
Extinction coefficient	0.0155(12)
Largest difference peak and hole (e Å^{-3})	1.778 and –1.478

[a] **$R = \Sigma(||Fo|-|Fc||)/\Sigma|Fo|$.** [b] **$R = [\Sigma w(|Fo|-|Fc|)^2/\Sigma w(|Fo|^2)]^{1/2}$, where $w=1/\alpha^2(|Fo|)$.**

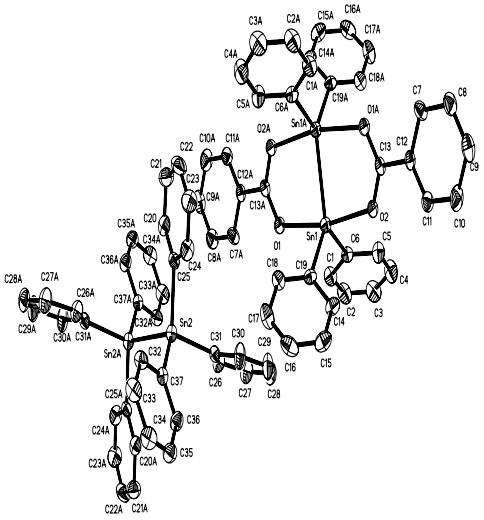


Figure 1. Molecular Structure of the Crystalline adduct

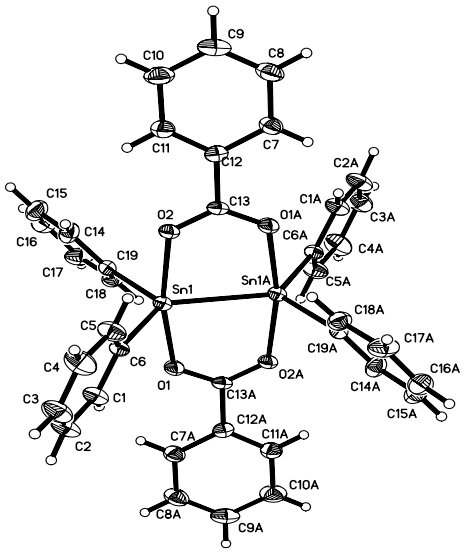


Figure 2. Molecular structure and atomic numbering scheme of A

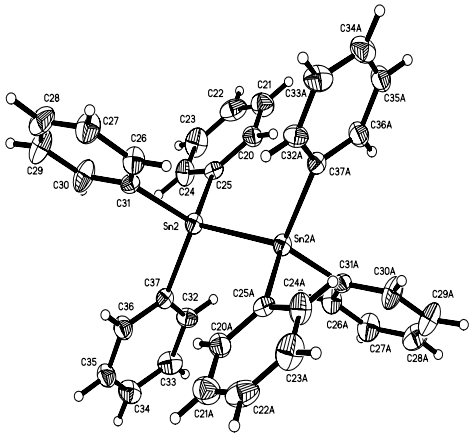


Figure 3. Molecular structure and atomic numbering scheme of B

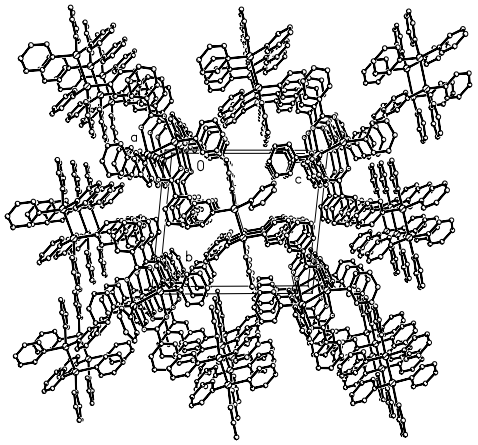


Figure 4. The crystal packing of the crystalline adduct

Table 2. Selected bond lengths (Å)

Sn(1)-C(6)	2.413(5)	C(14)-C(15)	1.367(9)
Sn(1)-C(19)	2.157(5)	C(15)-C(19)	1.383(8)
Sn(1)-O(1)	2.274(4)	C(15)-C(16)	1.397(11)
Sn(1)-O(2)	2.312(3)	C(16)-C(17)	1.358(12)
Sn(1)-Sn(1A)	2.6983(10)	C(17)-C(18)	1.421(9)
Sn(2)-C(31)	2.159(6)	C(18)-C(19)	1.361(8)
Sn(2)-C(37)	2.159(6)	C(20)-C(25)	1.345(9)
Sn(2)-C(25)	2.169(6)	C(20)-C(21)	1.432(11)
Sn(2)-Sn(2A)	2.8040(11)	C(21)-C(22)	1.385(12)
O(1)-C(13A)	1.285(6)	C(22)-C(23)	1.359(13)
O(2)-C(13)	1.265(6)	C(23)-C(24)	1.393(11)
C(1)-C(6)	1.396(8)	C(24)-C(25)	1.396(9)
C(1)-C(2)	1.408(9)	C(26)-C(31)	1.389(9)
C(2)-C(3)	1.362(11)	C(26)-C(27)	1.423(10)
C(3)-C(4)	1.374(12)	C(27)-C(28)	1.362(12)
C(4)-C(5)	1.383(10)	C(28)-C(29)	1.400(13)
C(5)-C(6)	1.395(8)	C(29)-C(30)	1.382(11)
C(7)-C(8)	1.411(9)	C(30)-C(31)	1.393(9)
C(7)-C(12)	1.415(8)	C(32)-C(33)	1.383(10)
C(8)-C(9)	1.375(10)	C(32)-C(37)	1.392(9)
C(9)-C(10)	1.397(11)	C(33)-C(34)	1.399(11)
C(10)-C(11)	1.402(9)	C(34)-C(35)	1.376(11)
C(11)-C(12)	1.362(8)	C(35)-C(36)	1.389(10)
C(12)-C(13)	1.503(7)	C(36)-C(37)	1.384(9)
C(13)-O(1A)	1.285(6)		

Table 3. Selected bond angles (º)

C(6)-Sn(1)-C(19)	115.3(2)	O(2)-C(13)-C(12)	117.9(5)
C(6)-Sn(1)-O(1)	93.1(2)	O(1A)-C(13)-C(12)	117.0(5)
C(19)-Sn(1)-O(1)	92.2(2)	C(15)-C(14)-C(19)	120.4(6)
C(6)-Sn(1)-O(2)	93.8(2)	C(14)-C(15)-C(16)	119.5(7)
C(19)-Sn(1)-O(2)	92.6(2)	C(17)-C(16)-C(15)	120.9(7)
O(1)-Sn(1)-O(2)	169.02(13)	C(16)-C(17)-C(18)	119.0(7)
C(6)-Sn(1)-Sn(1A)	123.39(15)	C(19)-C(18)-C(17)	119.7(6)
C(19)-Sn(1)-Sn(1A)	121.33(15)	C(18)-C(19)-C(14)	120.5(5)
O(1)-Sn(1)-Sn(1A)	84.88(10)	C(18)-C(19)-Sn(1)	119.7(4)
O(2)-Sn(1)-Sn(1A)	84.20(9)	C(14)-C(19)-Sn(1)	119.7(4)
C(31)-Sn(2)-C(37)	106.9(2)	C(25)-C(20)-C(21)	120.5(7)
C(31)-Sn(2)-C(25)	109.6(2)	C(22)-C(21)-C(20)	118.7(8)
C(37)-Sn(2)-C(25)	104.3(2)	C(23)-C(22)-C(21)	120.8(8)
C(31)-Sn(2)-Sn(2A)	111.5(2)	C(22)-C(23)-C(24)	119.7(8)

Continue Table3:

C(37)-Sn(2)-Sn(2A)	113.73(15)	C(23)-C(24)-C(25)	120.7(7)
C(25)-Sn(2)-Sn(2A)	110.4(2)	C(20)-C(25)-C(24)	119.5(6)
C(13A)-O(1)-Sn(1)	122.8(3)	C(20)-C(25)-Sn(2)	121.7(5)
C(13)-O(2)-Sn(1)	122.3(3)	C(24)-C(25)-Sn(2)	118.9(5)
C(6)-C(1)-C(2)	119.2(6)	C(31)-C(26)-C(27)	120.5(7)
C(3)-C(2)-C(1)	120.7(7)	C(28)-C(27)-C(26)	119.8(8)
C(2)-C(3)-C(4)	120.6(7)	C(27)-C(28)-C(29)	120.3(7)
C(3)-C(4)-C(5)	119.7(7)	C(30)-C(29)-C(28)	119.4(8)
C(4)-C(5)-C(6)	121.2(7)	C(29)-C(30)-C(31)	121.7(8)
C(5)-C(6)-C(1)	118.6(6)	C(26)-C(31)-C(30)	118.2(6)
C(5)-C(6)-Sn(1)	121.3(4)	C(26)-C(31)-Sn(2)	122.8(5)
C(1)-C(6)-Sn(1)	120.1(4)	C(30)-C(31)-Sn(2)	118.9(5)
C(8)-C(7)-C(12)	119.7(6)	C(33)-C(32)-C(37)	121.0(6)
C(9)-C(8)-C(7)	120.0(7)	C(32)-C(33)-C(34)	120.2(7)
C(8)-C(9)-C(10)	120.8(7)	C(35)-C(34)-C(33)	118.8(7)
C(9)-C(10)-C(11)	118.4(7)	C(34)-C(35)-C(36)	120.8(7)
C(12)-C(11)-C(10)	122.4(6)	C(37)-C(36)-C(35)	120.8(7)
C(11)-C(12)-C(7)	118.7(6)	C(36)-C(37)-C(32)	118.4(6)
C(11)-C(12)-C(13)	121.2(5)	C(36)-C(37)-Sn(2)	120.9(5)
C(7)-C(12)-C(13)	120.1(5)	C(32)-C(37)-Sn(2)	120.8(4)
O(2)-C(13)-O(1A)	125.1(5)		

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Study on the PPE Model Based on RAGA to Evaluating the Water Quality

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Abstract: This study improved the traditional genetic algorithm, and combined the new method named Real coding based Accelerating Genetic Algorithm (RAGA) with Projection Pursuit Evaluation (PPE) model. The RAGA-PPE model can optimize several parameters at one time. Based on this method, the authors built up a new evaluating model. Through applying the new model to evaluating nutrient states of south lake in Changchun, it gained the good results. Thus, we provided a new method and thought for readers who engage in researching the water quality evaluation. [Nature and Science. 2004;2(4): 34-38].

Key words: Real coding based Accelerating Genetic Algorithm (RAGA); Projection Pursuit Evaluation (PPE); water quality evaluation; model

1. Introduction

Water quality evaluation is to evaluate synthetically for the grade of water quality through building up mathematical model based on some evaluated indexes. Thus, we can provide some scientific gist for management and preventing pollution according to model. At present, there are many methods to evaluate the water quality. Such as gray clustering, fuzzy clustering, artificial nerve nets, (ANN) matter-element model and so on. (Jin, 2000). These models have the disturbance of giving weight by artificial factors and can't distinguish the grade precisely. Because the synthetic evaluation will be determined by many non-linearity indexes, when we build up model to classify and evaluate using traditional method, it is very difficult to find the internal rule. Recently, Friedme put forward a new arithmetic named Projection Pursuit (PP) which is fit for multivariate statistical analysis (Zhang, 2000). The kind of method can solve many non-linearity problems in a certain extent. But it is very difficult to find the best projection direction owing to the complicated space structure of multi-dimension data. Therefor, the author adopts Real coding based Accelerating Genetic Algorithm RAGA) to optimize the projection direction. RAGA can find the best value in the whole scope. Through using RAGA to reducing the dimension number, we can translate high-dimension data into the synthetic projection value in low-dimension sub-space. Thus, we can apply PPE model based RAGA to evaluate the water quality (Jin, 2000; Zhou, 2000).

2. Projection Pursuit Evaluation Model (PPE)

2.1 Brief introduction of PP model

The main characteristics of PP model are as follows. Firstly, PP model can handle the difficulty named dimension disaster, which have been brought by high-dimension data. Secondly, PP model can eliminate the jamming, which are irrespective with data structure. Thirdly, PP model provides a new approach to handle high-dimension problem using one dimension statistics method. Fourthly, PP method can deal with non-linearity problem (Jin, 2000; Zhang. 2000).

2.2 Step of PPE modeling

The step of building up PPE model includes 4 steps as follows (Jin, 2000; Zhang. 2000).

Step 1: Normalizing the evaluation indexes set of each sample. Now, we suppose the sample set is $\{x^*(i,j)|i=1\sim n, j=1\sim p\}$. $x^*(i,j)$ is the index value of j and sample of i. n ——the number of sample. p ——the number of index. In order to eliminate the dimension influence and unite the change scope of each index value, we can adopt the following formulas

to normalize the data.

$$x(i,j)=\frac{x^*(i,j)-x_{min}(j)}{x_{max}(j)-x_{min}(j)} \quad (1—a) \text{ or:}$$

$$x(i,j)=\frac{x_{max}(j)-x^*(i,j)}{x_{max}(j)-x_{min}(j)} \quad (1—b)$$

In formula: $x_{max}(j)$ and $x_{min}(j)$ stand for the max and the min of j index value. $x(i,j)$ is the index list after moralization.

Step 2: Constructing the projection index function $Q(a)$. PP method is to turn p dimension data ($\{x^*(i,j)|j=1\sim p\}$) into one dimension projection value $z(i)$ based on projection direction a.

$a=\{a(1),a(2),a(3),\cdots,a(p)\}$,

$$z(i)=\sum_{j=1}^{p}a(j)x(i,j) \quad (i=1\sim n) \quad (2)$$

Then, we can classify the sample according to one-dimension scatter figure of $z(i)$. In formula (2), a stand for unit length vector.

Thus, the projection index function can be expressed as follows.

$$Q(a)=S_z D_z \quad (3)$$

In formula: S_z ——the standard deviation of $z(i)$, D_z ——the partial density of $z(i)$.

$$S_z=\sqrt{\frac{\sum_{i=1}^{n}(z(i)-E(z))^2}{n-1}} \quad (4)$$

$$D_z=\sum_{i=1}^{n}\sum_{j=1}^{n}(R-r(i,j))\cdot u(R-r(i,j)) \quad (5)$$

In formula (4) and (5), $E(z)$ ——the average value of series $\{z(i)|\,i=1\sim n\}$; R ——the window radius of partial density, commonly, $R=0.1S_z$. $r(i,j)$ ——the distance of sample, $r(i,j)=|z(i)-z(j)|$; $u(t)$ ——a unit jump function, if $t\geq 0$, $u(t)$ =1,if $t<0$, $u(t)$ =0.

Step 3: Optimizing the projection index function. When every indexes value of each sample have been fixed, the projection function $Q(a)$ change only according to projection direction a. Different projection direction reflects different data structure characteristic. The best projection direction is the most likely to discovery some characteristic structure of high-dimension data. So, we can calculate the max of $Q(a)$ to estimate the best project direction.

Function: $Max: Q(a)=S_z\cdot D_z$ (6)

Restricted condition $s.t: \sum_{j=1}^{p}a^2(j)=1$ (7)

Formula (6) and (7) is a complex non-linearity optimization, which take $\{a(j)|\,j=1\sim p\}$ as optimized variable. Traditional method is very difficulty to calculate. Now, we adopt RAGA to handle the kind of problem.

Step 4: Classification. We can put the best projection direction a^* into formula (2), then we can obtain the projection value of each sample dot. Compare $z^*(i)$ with $z^*(j)$. If $z^*(i)$ is closer to $z^*(j)$, that means sample i and j are trend to the same species. If we dispose $z^*(i)$ from big to small, we can obtain the new sample list from good to bad.

3. Real coding based accelerating genetic algorithm (RAGA)

3.1 Brief introduction of GA

Genetic Algorithm has been put forward by Professor Holland in USA. The main operation includes selection, crossover and mutation (Jin, et al. 2000; Zhou, et al. 2000).

3.2 Real coding based accelerating genetic algorithm (RAGA)

The coding mode of traditional GA adopted binary system. But binary system coding mode has many abuses. So, through consulting literature(Jin, 2000), the author put forward a new method named RAGA (Real coding based Accelerating Genetic Algorithm). RAGA includes 8 steps as follows. For example, we want to calculate the following best optimization problem.

$$Max: \text{f(X)}$$
$$\text{s.t.}: a_j \leq x_j \leq b_j$$

Step1: In the scope of $[a_j,b_j]$, we can create N group uniformity distributing random variable $V_i^{(0)}(x_1,x_2,\cdots x_j,\cdots x_p)$. $i=1\sim N$, $j=1\sim p$. N——the group scale. p —— the number of optimized parameter.

Step 2: Calculate the target function value. Putting the original chromosome $V_i^{(0)}$ into target function, we can calculate the corresponding function value $f^{(0)}(V_i^{(0)})$. According to the function value, we dispose the chromosome from big to small. Then, we obtain $V_i^{(1)}$.

Step 3: Calculate the evaluation function based on order expresses as $eval(V)$. The evaluation function gives a probability for each chromosome V. It makes the probability of the chromosome to be selected is fit for the adaptability of other chromosomes. The better the adaptability of chromosome is, the much easier to be selected. Now, if parameter $\alpha \in (0,1)$, the evaluation function based order can be expressed as follows.

$eval(V_i) = \alpha(1-\alpha)^{i-1}$, i = 1,2,…, N

Step 4: Selecting operation. The course of selecting is based on circumratating the bet wheel N times. We can select a new chromosome from each rotation. The bet wheel selects the chromosome according to the adaptability. We obtain a new group $V_i^{(2)}$ after selecting.

Step 5: Crossover operation. Firstly, we define the parameter P_c as the crossover probability. In order to ensure the parent generation group to crossover, we can repeat the process from $i = 1$ to N as follows. Create random number r from [0，1]. If $r < P_c$, we take V_i as parent generation. We use $V_1^{'}, V_2^{'}, \cdots$ to stand for male parent which to be selected. At the same time, we divide the chromosome into random pair based on arithmetic crossing method. That is as follows.

$X = c \cdot V_1^{'} + (1-c) \cdot V_2^{'}$ $\quad Y = (1-c) \cdot V_1^{'} + c \cdot V_2^{'}$

c ——a random number from (0,1).

We can obtain a new group $V_i^{(3)}$ after crossover.

Step 6: Mutation operation. Define the P_m as mutation probability. We select the mutation direction d randomly from R^n. If $V + Md$ isn't feasible, we can make M a random number from 0 to M until the value of $V + Md$ is feasible. M is a enough big number. Then, we can use $X = V + Md$ replace V. After mutation operation, we obtain a new group $V_i^{(4)}$.

Step 7: Evolution iteration. We can obtain the filial generation $V_i^{(4)}$ from step 4 to step 6, and dispose them according to adaptability function value from big to small. Then, the arithmetic comes into the next evolution process. Thus, the above steps have been operated repeatedly until the end.

Step 8: The above seven steps make up of Standard Genetic Arithmetic (SGA). But SGA can't assure the whole astringency. The research indicates that the seeking optimization function of selecting and crossover has wear off along with the iteration times increasing. In practical application, SGA will stop to working when it is far away from the best value, and many individuals are conform or repeated. Enlightening by reference (Jin, 2000), we can adopt the interval of excellence individual during the course of the first and the second iteration as the new interval. Then, the arithmetic comes into step 1, and runs SGA over again to form accelerate running. Thus, the interval of excellence individual will gradually reduce, and the distance is closer to the best dot. The arithmetic will not stop until the function value of best individual less than a certain value or exceed the destined accelerate times. At this time, the currently group will be destined for the result of RAGA.

The above 8 steps make up of RAGA.

3.3 PPE model based on RAGA

Take projection function $Q(a)$ as the most target function in the PPE model and the projection $a(j)$ of each index as optimized variable. Through running 8 steps of RAGA, we can obtain the best projection direction $a^*(j)$ and projection value $z(i)$. Compare the $z(i)$ each other, we can obtain the evaluated result. Then, through comparing the distance between $z(i)$ and $Z(i)$, the smallest distance between any two samples, then, the number i is the soil sample grade.

4. Application example

Now, we use the data of literature [5] (Lu, 1999) to build up the RAGA-PPE model of water quality evaluation. The evaluated standards of the nutrition degree in lake see also to Table 1.

Table 1. Evaluation standard of lake nutrient states

Grade	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ	Ⅶ	Ⅷ
Nutrition style	**Poor nutrition**	**Poor –medium nutrition**	**Medium nutrition**	**Medium-rich nutrition**	**Rich nutrition**	**Rather rich nutrition**	**Quite rich nutrition**	**Abnormity rich nutrition**
Chemistry oxygen demand（mg/L）	0.48	0.96	1.80	3.60	7.10	14.0	27.0	54.0
Total nitrogen（mg/L）	0.079	0.16	0.31	0.65	1.20	2.30	4.60	9.10
Total phosphor（mg/L）	0.0046	0.01	0.023	0.05	0.11	0.25	0.56	1.23

We can build up the PPE model of the evaluation standard about the nutrition states in lake based on MATLAB 5.3.

During the course of RAGA, the parent generation scale is 400 ($n=400$). The crossover probability is 0.80 (p_c =0.80). The mutation probability is 0.80 (p_m =0.80). The number of excellence individual is 20. α =0.05. Through accelerating 9 times, we can obtain the best projection value. That is 0.4203. The best projection direction: a^* =（0.5634，0.5553，0.6117）. Putting a^* into formula (2), we can obtain the projection value of each villages and towns. That are $z^*(j)$=（0.0121，0.0248，0.0491，0.1021，0.2020，0.4107，0.8409，1.7304）(Figure1, Table 2).

The PPC model based on RAGA of water quality is as follows.

$$y^*(i)=1.4138\ln z^*(i)+7.243 \qquad R^2=1.0000$$

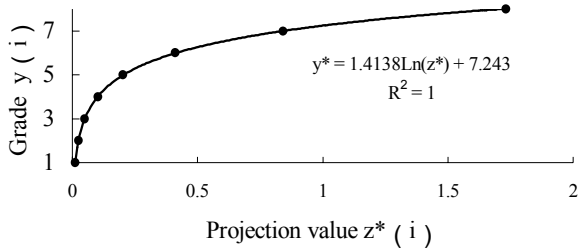

Figure1. The relation between water quality projection value and grade.

Table 2. Analyzing the error of PP model.

Experience value	1	2	3	4	5	6	7	8
Calculated value	1.0017	2.0163	2.9820	4.0170	4.9816	5.9849	6.9980	8.0183
Absolute error	0.0017	0.0163	-0.018	0.017	-0.0184	-0.0151	-0.0020	0.0183
Relative error	0.17%	0.815%	-0.60%	0.425%	-0.368%	-0.252%	0.029%	0.229%

The average absolute error is 0.0134, and the average relative error is 0.361%. We can see that the fit precision of RAGA-PPC model is rather high. So the PPE model can describe the relation between the evaluated index and water grade.

Now, we calculate the projection value of water quality about South-lake in Changchun during the course of June 1997 to May 1998 (Table 3).

Table 3. Evaluation results of nutrient states of South Lake water in Changchun.

Time	Chemistry oxygen demand（COD）（mg/L）	Total nitrogen（TN）（mg/L）	Total phosphor（TP）（mg/L）	BP Model[5]	Projection value Z*	Calculated value y*	RAGA—PPE Evaluated grade
1997.6	39.63	6.38	0.25	Ⅷ	1.0131	7.2614	Ⅶ
1997.7	40.73	2.85	0.36	Ⅷ	0.8682	7.0431	Ⅶ
1997.8	23.15	9.56	0.16	Ⅶ	0.9363	7.1499	Ⅶ
1997.9	35.24	5.76	0.13	Ⅷ	0.868	7.0429	Ⅶ
1997.10	32.42	2.40	1.15	Ⅷ	1.0531	7.3161	Ⅶ
1997.11	17.35	2.08	0.52	Ⅵ	0.5706	6.4498	Ⅵ
1997.12	40.67	2.05	0.63	Ⅷ	0.9317	7.1430	Ⅶ
1998.1	41.36	2.92	0.03	Ⅷ	0.7453	6.8273	Ⅶ
1998.2	40.19	4.50	1.49	Ⅷ	1.4205	7.7393	Ⅷ
1998.3	25.72	3.16	1.30	Ⅷ	1.0676	7.3417	Ⅶ
1998.4	33.32	2.57	1.01	Ⅷ	1.0178	7.2679	Ⅶ
1998.5	36.89	4.88	0.37	Ⅷ	0.9379	7.1524	Ⅶ
Year Average	32.35	4.83	0.62	Ⅷ	0.9758	7.2084	Ⅶ

From Table 3 we can know that the water quality in November, 1997 belongs to grade Ⅵ, and in February, 1998 belongs to grade Ⅷ during 1996/6 to 1998/5. The other months belong to grade Ⅶ. The

water quality in the whole year belong to grade Ⅶ. These mean the South-lake is in the states of graveness rich nutrition. The varied rule is rather accord with the practical condition. The PPE model has the same varied trend as the BP mode applied in literature (Lu, 1999). The time of wave crest and trough are the same. The results of other 10 months are litter higher than PPE model based on RAGA. The reason is obviously. When we are training the evaluated standard with BP network, there are artificial disturbance and subjectivity of determining the expected grade value output by BP network. Furthermore, because the distinguished ability of the BP network is rather low, so the result isn't accord with the RAGA-PPE model. But both of the two models can reflect the practical condition.

Furthermore, the best projection direction can reflect the influential degree of water quality grade caused by every evaluated index. The projection value is bigger, and it will have much influence for water quality evaluation. Thereby, we can verify the reasonability of the water quality evaluated standard. In the example, the best projection direction is a^* = （0.5634，0.5553，0.6117）. That means the three evaluated indexes have the same function in evaluating the water quality.

5. Conclusion

(1) Through applying PPC model, the author builds up the PPE model of evaluating the water quality. Several evaluation indexes have been taken as multi-dimension projection parameters to seeking the best projection direction. The best projection index function value can reflect the quality of each soil sample good or bad. Thus, we can avoid the disturbance by artificial factor to endow weight. The result is good.

(2) The author improves on SGA, and put forward a new method named RAGA through reducing the interval of excellence individual to accomplish the accelerate process. Thus, the method of RAGA can realize quick convergence and seeking the best result in the whole scope.

(3) Combing RAGA with PPE model, through using RAGA to optimizing the many parameters in the PPE model, we can obtain the best projection direction of evaluation index of each sample. Thus, the process of PPE modeling has been predigested. And the PPE model can be used in many other fields.

(4) The author put PPE model based on RAGA into the region of water quality evaluation. The PPE model can reflect the corresponding relation of non-linearity between classification number and projection value. The grade has been divided in focus. The precision of model is high. Furthermore, the best projection value can reflect the influential degree of each index during the course of total evaluation. Thus, the author provide a new method and thought for researching country energy programming.

Acknowledge

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The Creation of Management System, Water Property Rights and Economic Results Research of the Small Irrigative Regions in China

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Abstract: At present, the management system falls behind, the system creation is not enough and the water property rights are indefinite, which result in low efficiency of water utilization in the irrigative regions of China. Indefinite property rights caused unreasonable installs of the resource, waste, abusing and the occurrence of low efficiency. Based on these problems, by the reaches and studies on Changgang Irrigative Region of Lanxi county of Heilongjiang Province of China, which changed the management mechanism of water expenditure. This paper builds up the economic analysis on the agriculture irrigation efficiency. It made the conclusion that economic results will greatly improved by building water market. At the same time, only management system continuously reformed can ensure the effective mechanism of the irrigative regions in good operation. [Nature and Science. 2004;2(4):39-48].

Key Words: system creation; water property rights; resources installs

Introduction

China is a large country in agriculture in the world, also is a large country in irrigation. In the gross amount of water resource, the agriculture irrigation takes a lion' part. In 2000, the national agriculture water consumption takes 80% in the gross amount. Among it, the irrigation takes 67%. On agriculture planting, China regards supplementary irrigation as the lord, the whole country demand supplementary irrigation 1.23 m^3, while producing 1 kg corns. But the dense population caused personal owning is extremely limited .The northern farmland area takes 3/5 of the national farmland area, but the water resource is only 1/5. On the contrary, the southern farmland area takes 2/5 while the water resource is 4/5. The southern farmland has water 28695 m^3/hectarn; while the northern has only 9465 m^3. There are 15 provinces (area) whose amount of water can't reach to 1500 m^3/hectarn. Among them, 13 are in the north. Especially the recent years, on the short of the water resources, the agriculture irrigation has suffered rigorous challenge. At the same time, owning to backward in management system, lots of irrigative regions faced up the problems: water fee can't hand in, water consumption can't get guaranteed, the peasants aren't positive, engineering is in aging and bad repair, the beneficial results drop. Therefore, solving is utmost urgency. Changgang Irrigation Region of Lanxi County, Heilongjiang Province of China changed the management mechanism of water consumption; explicated water property rights. In 3 years, the beneficial taking a favorable turn, and farmer's income level obviously increased.

1. The basic condition and problems of Changgang Irrigative Region

Changgang Irrigative Region of Lanxi country Heilongjiang Province set up in 1964.The designing irrigation area is 1,500 acre. The irrigative region management-station is a business unit, has 19 workers. Our country subsidizes 17 thousand yean annually. In 1980, bad management forced to ceasing irrigation. From 1989 to 1997, the irrigative region proceeded maintaining the operation while doing the patchable reform. But there's difficulty in collecting the fees, the management section is lots off debts, the equipments were aging the engineering was in bad repair and the management fell behind. So it ceased irritation again is 1998. Then the equipments in the pump-station were idle, the workers were unemployed. 1.8 thousand farmers of 647 households who relied on the irrigative region can't reach their wishes that growing the crops, living a life of reasonable comfort. They appealed many rimes for their extremely difficult lives. Like

this, the ten thousand acre irrigation area, which invested 5,000,000 yean, was sunk in paralytic.

The above problems suffered the Country party committee and the Country Government. To discard the irrigative region thoroughly or leave no stone unturned to get away from the predicaments, is not only the dilemma of the leaders at different levels, but also the disputed topic of all the people. Lanxi Country Party Committee, Country Government has the courage to renovate. They realized that, in nowadays, transforming the traditional water conservancy into resources water modern water conservancy, transforming the engineering water conservancy into resources water conservancy, it must solve the problem that the reformation of management system, productive relation in rural area can not adapt the market economy. It must solve the antinomy between the marketing of water merchandise and public welfare of irrigative management. It must solve the antinomy between the account of water fee and lasting development of the irrigation area. It must solve the antinomy between the engineering construction and the lack of the investment.

In November 1999, Lanxi County Conservancy Bureau asked for the upper supervisory section, and listened to the farmer's opinions, by investigating and studying over and again. They put forward the contracted reformation project about the Changgang Irrigation Area, which handed in and approved by the County Party Committee, County Government. They agreed in bid in the water conservancy interior. On April 1rt, 2000, Fan Chun comrade, a senior engineer of the County Water Conservancy Bureau wined in the bid, signing the contract with the supervisory section. Changgang Irrigation Area uncovered a new page heading to the new century finally.

What Changgang Irrigation Area practice is the zero price-contract, the contractor didn't hand in any fee to the Water Conservancy Bureau. And the contracted period is 25 years. During the period, the augmentative engineering and equipments by maintaining the building should give to the first party unconditionally. The contractor must employ the current workers. The contractor has the power to manage and use but can not realize the all properties. The funds of maintaining the equipments and the engineering are solved by the contractor himself. It must ensure to materialize standardization of the Irrigation Area and act. It provided definitely the both parties' power, responsibility, benefit in the contract.

After the contract, the operating nature of Irrigation Area changed, transforming government-owned into public-operation. A fresh and live blood infused into the muscle, which was on the verge of decadent. A series remarkable changes took place in the management of the Irrigation Area. The contractor raised funds 410,000 yean to repair the engineering, prepaid the electricity charge 2,050,000 yean, recovered thee irrigation, prevented leakage in the leaking channels, renovated the patchable engineering in the farmland, enlarged the irrigation areas. The stationmaster was appointed, although the old casts were employed, they have one chance to contest the post. Wage-reformation was practiced. The monthly wage of the station –master is 1,500 yean, and the senior clerk' is 1,200 and the manage personnel' is 800. Award and publish system on saving water practiced strictly. The enforcement of the reformation measures like a delight flowing water, moistening the workers and the farmers' hearts. The same year, the Irrigation Area recovered 333.3 hectares, and increased aqueous-merchandise' value. The water consumption per 1 acre from 1700 m^3 reduced to 1100 m^3. Owing to non-water-matter dispute, the farmers no longer sit in the ground to wait water, but were told "water comes" at the midnight by the workers. Never wished to hand in the water fee, then helped the workers to collect the water fee, cried to demand the water, then killed the pigs and goats to appreciate supplying water on time. Changgang Irrigation Area and the farmers who counting on it are all changed.

2. The system-economic analysis on the reason of low efficiency in agriculture irrigation

2.1 The water property rights is misty

That a property right is definite is the basic term of the market mechanism retaining normal operation. It's the precondition to exploit, exchange, keep, manage the resources and invest with resource, without exception of the water resources. Firstly, indefinite water rights result in weak exterior control of the water expenditure. The obtaining water quantity mainly under the yoke of the natural factors. Especially the upper reaches' exploitation is usually above plan, and the waste is serious .The widespread phenomenon of "pump water-contest" is existed. Secondly, indefinite water rights result in the low effectiveness of resources install. The property rights

indefinite, then the water is non-negotiable, and water resources can't be flown from low-value realm to costliness realm, which lowered the national gross beneficial result. In fact it's a distortion of resources install.

2.2 The price of water is low (Table 1)

For long times, the standard of water fee is low. The difference between water cost and the government subsidizes water price. On one hand, in people's opinion that water is a priceless resources and they make the habit of wasting water in production activity and livelihood. On the other hand, the investment to agriculture hydraulic engineering can't be recovered on compensation, then the national support is increased. The water-supply engineering is aging and hard to maintain. What make it worse, during the period of economic system reformation, the government subsidy decreased year by year? The funds of the Irrigation Area management unit operated difficultly.

2.3 The governors are pursuing political achievements in their tenure

The irrigation system's construction and management are invested by the government. The national wage and subsidy are the source of the operated governors, not the service quantity that provided to the farmers. The governors' tenure limit and the post promotion system make them preferred to keep good relations with superior and keep the short-term performance of the irrigation system. Once the governors' work was only for pleasing the superior but not satisfying the farmers' demand, they would not have enough motives to obtain the extensive and accurate information. The governors were encouraged by looking for funds to develop a new irrigation system, but didn't safeguard the original irrigation system carefully. Developing a new irrigation system is easier than safeguarding the original irrigation system on expressing themselves at the political achievement in their tenure. They would be appreciated by the superior and promoted the post easier. During the period of 1985-1995, the governors of Changgang Irrigation Area changed 5 times. Their short-term behaviors made serious loss to the collecting economy.

Table 1. The price of water, water cost, subsidy rate in different periods of Changgang Irrigation Area

Term	1990	1995	1997
The price of water (yuan/m^3)	0.020	0.022	0.025
Water-cost (yuan/m^3)	0.030	0.040	0.047
Subsidy rate (%)	18.0	8.0	0

2.4 The management system can't adapt to the objective request of reformation and development in the Irrigation Area

Like most small irrigation areas in current, Changgang Irrigation Area belongs to the nation, no change in 50 years. The County (municipal) Water Conservancy Bureau leaded the management-unit. The governors and the farmers, the County Government, and the Village Party Committee didn't handle matters at common benefits, which made the farmers have no enthusiasm too participate the management. On the water fee paying, especially in the droughts, they had conflicted motion. The Irrigation Area entrusted the County Government or the Village Party Committee to collect the water fee, which created chances for some officials' "attempt rent" behavior. The farmers contained lingering fear. Therefore, to encourage the farmers to participate in the water expenditure management and the development, It should be leaded by the market economy, to give full play to the governors, the farmers, the County Government, and the Village party Committee's enthusiasm and creativeness on the water expenditure management.

3. The economic analysis on the irrigative water expenditure efficiency

The improvement of the Changgang Irrigation Area' water expenditure efficiency, is attributed to the effective operation in practice and the guidance on

theory. Under the following 4 different kinds condition, we discuss the farmers' profit. Draw a conclusion: that by establishing water (although be prepared), according to m^3 to collect the fee, and the saved water resources be made over, do favor in saving water and bringing economic results into play (Figures 1-4). The following is the concrete analysis:

3.1 Supposes on the terms

(a) The farmer is an economic person, pursuing the profits-maximize. The farmer owns land area T. The land can't be made over, planting a certain kind crop.

(b) In the process of practice irrigation, the water loss is G (including the farmland evaporation, underground permeation, and the loss in the irrigation management). This part of loss is unavoidable, only can be reduced by taking some certain water saving measures. The water supplying ' loss is Go. While there's no water saving investment .The saved water is g when there has water saving investment. Water saving investment is I. I is the function of g's .

The $G=Go-g$

$I=I(g)$ $(I'>0, I''>0)$

(c) The water demand of the farmer is X .X=G+W. The W is the water demand of natural growing. Suppose this land has abundant water, the farmer can acquire the whole demand, and his water consumption won't exceed his allotted water Xo. Then the surplus water is Xo-X.

(d) This crop' output is Q. It's production function is Q.Q=Q (W, T), that is a kind function of variable factors, i.e. the devotion of water is variable. Suppose the devotion W and the output Q present the regulation that the income decreased gradually. This crop' unit yield is P.

(e) The farmer can't affect the water price, but can affect the profits by choosing the yield level.

3.2 Collecting the water fee is according to the land area. The water price of unit area is Po, and the water-trade is nonexistent (Figure 1).

In this circumstance, the farmer considered the price of 1 m^3 is zero. So he will not take any cost-means. The water expenditure balanced term that the farmer pursuing the profits-maximize is:

$\Pi 1=$ (sales income) – (the expense of water expenditure) – (the cost of water saving)

$=P*Q(W,T)-P_o*T-I(g)$

The water expenditure balanced term that the farmer pursuing the profits-maximize is:

$\Pi_{1w}=P*Q_w=0$

$\Pi_{1g}=I'=0$

i.e. when the farmers' profits reach maximize , water expenditure' limit performances is equal to water saving' limit cost , is zero. Water expenditure' limit performances is the change of the output which droved by increasing a unite water. Water saving' limit cost is the wasted cost for the farmer to save a unit water.

$\Pi 1=$ (sales income) –(the expense of water expenditure) –(the cost of water saving)

$=S_{oaw}-P*T$ (such as diagram (1) shows, the S means the area)

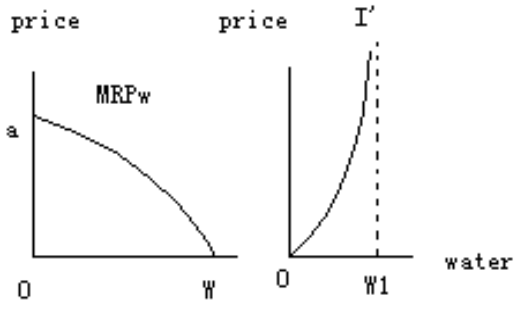

Figure 1. Collecting fees according to the area, water-trade market is nonexistent, the farmer' profit sketch map (1)

In diagram (1), the OW means the crop' water consumption, OW_1 means the loss G_o while taking no water saving measures. The water saving measures "g" is zero.

3.3 Collecting the water fee according to the land area. The water price of the unit area is Po. Water-trade market is existed. The water bargain price of 1 m^3 is Pa (Figure 2).

In the assumption, the farmer would like to sell a part or all part of his allotment to non-agriculture section. In this circumstance, the farmer will take some necessary measures to save water. Use the $\Pi 2$ to mean the farmer' profits in this circumstances.

$\Pi 2=$(sales income) +(income of selling water) –(the expense of water expenditure) –(the cost of water saving)

$=P*Q(W,T)+P_a*(X_o-W-G_o+g)-P_o*T-I(g)$

The water expenditure balanced term that the farmer pursuing the profits-maximize is:

$\Pi_{2w}=P*Q_w-P_a=0$

$\Pi_{2g}=P_a-I'=0$

i.e. water expenditure' limit performances =water saving' limit performances = the bargain price of water

Π_2=(sales income) +(income of selling water)–(the expense of water expenditure) –(the cost of water saving)

$=S_{oabw}+(S_{wbcw3}+S_{0dew2}+S_{w1fgx0})-S_{opabw}-S_{oew2}$ (such as diagram (2) shows)

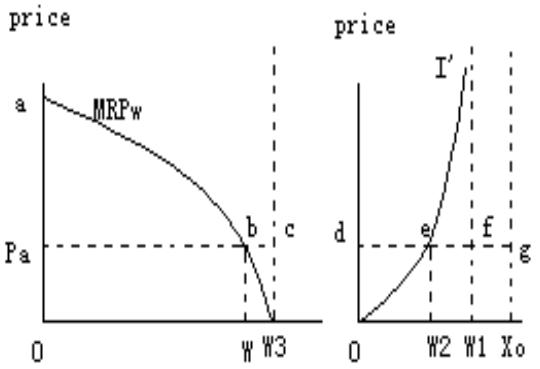

Figure 2. Collecting fees according to the area, water-trade market is existed, the farmer' profit sketch map (2)

In diagram (2), the OW means the crop actual water consumption. The WW_3 means the reduced water consumption .The W_1X_0 means surplus water even there is no water saving, i.e. X_0-G_0 .The OW_2 means the saved water "g" by taking water saving measures. The W_1W_2 means the loss G in actual irrigation process.

3.4 Collecting water fee P_w according to 1 m^3. Water-trade market is nonexistent (Figure 3).

In this circumstances, coming from the mental state that to decrease the planting cost, the farmer will adopt a certain water saving measures. Use the Π_3 means the farmer' profit in this circumstances.

Π_3=(sales income) –(the expense of water saving expenditure) –(the cost of water saving)

$=P*Q\ (W,t)-P_W*(W+G_o-g)-I(g)$

The water expenditure balanced term that the farmer pursuing the profits-maximize is:

$\Pi_{3w}=P*Q_w-P_w=0$

$\Pi_{3g}=P_w-I'=0$

The conclusion is, $P*Q_w=P_w=I'$, i.e. for the sake of production, the farmer' devoted in water saving, until the water' limit performances is equal to the limit expense of water saving.

Π_3=(sales income) –(the expense of water saving expenditure) –(the cost of water saving)

$=S_{oabw}-(S_{opwbw}+S_{efw1w2})-S_{oew2}$

Such as diagram (3) shows, the OW means the crop' water consumption. The WW_3 means the reduced water. The OW_2 means the saved water "g" by adopting the water saving measures. The W_1W_2 means the loss G in actual irrigative process.

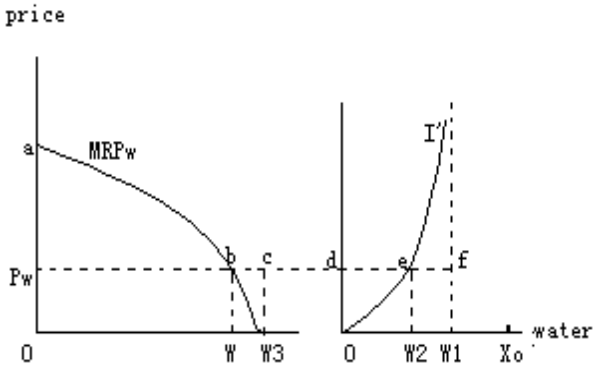

Figure 3. Collecting fees according to the area, water-trade market is existed, the farmer' profit sketch map (3)

3.5 Collecting water fee P_W according to 1 m^3. Water-trade market is existed. The bargain price is Pa per 1 m^3, and $P_a>P_w$ (Figure 4)

In the assumption, the farmer would like to sell a part or all part of his allotment to non-agriculture section. In this circumstance, the farmer will take some necessary measures to save water. Use the $\Pi 4$ to mean the farmer' profits in this circumstances.

Π_4 = (sales income) + (income of selling water) – (the expense of water expenditure) – (the cost of water saving).

$=P*Q\ (W,T)+P_a*(x)-W-G_o+g)-P_w(W+G_o)-I(g)$

The water expenditure balanced term that the farmer pursuing the profits-maximize is:

$\Pi_{4w}=P*Q_w-P_a-P_w=0$

$\Pi_{4g}= P_a -I'=0$

The farmer's choice on planting strategy is point b at water price Pa+Pw. The corresponding water consumption is W. But the farmer' actual cost on planting strategy is point g at the water price Po. The saved water was all used to trade.

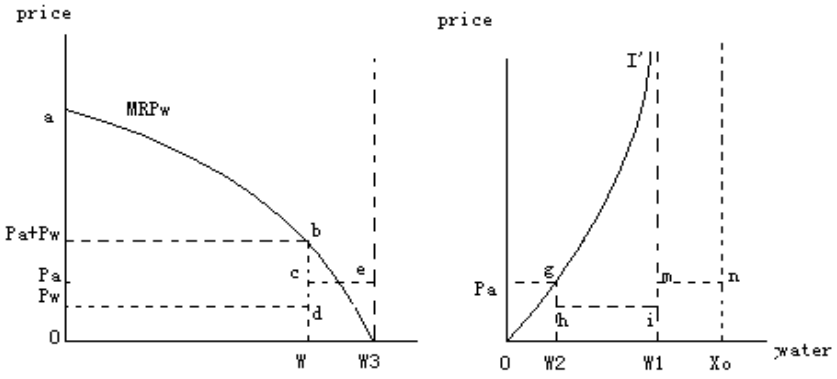

Figure 4. Collecting fees according to the area, water-trade market is existed, the farmer' profit sketch map (4)

$\Pi 4$=(sales income) +(income of selling water) –(the expense of water expenditure) –(the cost of water saving)

$=S_{oabw}+(S_{wcew3}+S_{opagw2}+S_{w1mnx0})-S_{opwdw}-S_{w2hiw1}-S_{ow2g}$

In diagram (4), the OW means the crop actual water consumption. The WW_3 means the reduced water consumption. The W_1X_0 means surplus water even there is no water saving, i.e. X_0-G_0 .The OW_2 means the saved water g by taking water saving measures. The W_1W_2 means the loss G in actual irrigation process.

We can get the conclusions from the above model analysis: $\Pi_1<\Pi_2, \Pi_3<\Pi_4$. By water property rights market, on one hand, along with the proceeding of the adjustment on agriculture industrial structure, the farmer will replant a new crop what in low coefficient on water expenditure or efficiently crop. Otherwise, its water resource limit performance will be lower than the price of the water resource that made over. The income from making over the water resource by water rights market, also can be used to complement the shortage of the infrastructure funds on farmland and irrigation, quicker the construction of water saving irrigative equipment, to form virtuous cycle of the work on agriculture water saving.

4. System creation on management of water expenditure in irrigation area

4.1 The establishment of the water rights

Are water rights problems existed or not in Changgang Irrigation Area? What advantages can bring in by defining and explicating the water rights? The governor of Changgang Irrigation Area just knew the concept of "water rights" at the end of 2001, and the farmer knew the proceeding on explicating "water rights" in the spring of 2002. The main "water rights" problems to solve were: how to handle the remained water? The handling can benefit the water saving and the farmer or not? To solve these problems, should it definite the power on profit and making over with the remained water or not?

In <<The water laws>> rules: "The water resources belong to the nation, i.e. owned by all the people". "All the ponds, the water in the reservoirs which owned by agriculture collective economy, belong to the collective." Water expenditure in irrigation from half-open exploitation to exercises of collective water rights, what is the basic direction of water rights vicissitudes. The basic meaning of water rights of irrigation area turned into collective water rights is: to give the more definite power on usage and profit, and the suitable power to make over is allowed. At the angle of management, the irrigation area water rights is the power to give the particular irrigation area a certain quantity and quality water resources which is decided by the public structure or public represents with form of organization and consultation, singing the agreement, on the irrigation area itself, water rights means the irrigation area which regarded as water expenditure group owning the power to acquire and consume a certain quantity and quality water resources in a certain resources valley or region. It decided by the factors of its population quantity, resources condition, and potential development act. At the same time, we should notice that, the water rights of irrigation area is owned by all the residents, and entrusted to the public structure to manage. The irrigation area should have a initial total water consumption, i.e. the initial water rights. The irrigation area has the power to benefit and make over with the remained water. Laws is the most common method to definite the property rights of nature resources,

without exception of the water resources. The intervention of the laws can reduce the bargain expenses by building the scaled economy that the property rights is defined.

To definite the water rights, the concrete ways of Changgang Irrigation Area:

Firstly, practiced the water-trade. Establishing the water supplied by water conservancy engineering belongs to the characteristic merchandise, building the business relations between the buyer and the seller. The management unit of the irrigation area supplied water according to the contract. The farmers consume the irrigative water by paid. In a questionnaire, almost all the farmers knew that water is merchandise. 2/3 can speak out the current water price and the price policy. All the farmers advanced a part of the water fee at the beginning of a year.

Secondly: the farmers participated in the management directly. The Water Expenditure Association request the farmers to participate the management what is all-directions, including the investment and the construction, maintenance and management on the farmland irrigation engineering in the magistracy of the Water Expenditure Association. Also, they participate in formulating the water expenditure plan supplying the water, supervising democracy with the association and the water-supplying section. An investment shows that, the mainly express that the farmers participate in the management lies in the formulation of water expenditure plan, democracy supervision positively. The Water Expenditure Association increased the openness of democracy decision. They let the farmers know the election to the association director, wage-paid and the management expenses share the water fee.

The farmers know that, the purpose of participating in the association positively is to make their opinions adopted. The wishes that increase their quality of themselves are enhanced continuously.

Thirdly, Water Expenditure Association and the development of agriculture economy have already displayed the positive interaction the establishment and maintenance of the channels, the timeliness of water supplying, democracy supervision, the relations between the association and the water conservancy section and act, these aspects are all increased. Thus, the water management section decreased the inappropriate interfere to the irrigation activities. The reasonable burdens of the farmers are alleviated. The property rights have a certain definition. The society bargain cost descended. And the development of the agriculture economy is speeded.

Finally, the benefits compensation mechanism is established. The core of the benefits compensation mechanism is to guarantee the farmer' benefits, especially the benefits of the poverty households and the injured person, to bring about the encouragement on water saving to the farmers. Water saving can compensate for a loss of less expenditure completely, and also for the cost by the increased water price. Comparing with the original much expenditure, the farmers can acquire more income. Then it can attain the dual purpose that building efficient consumption of water for agriculture and benefiting the farmers. With the support of the government, the encouraging measures to the Water Expenditure Association and the farmers on water saving is practiced. The channels compensating the benefits are: the public finances transferring paid, the income of water trade, and the national devotion and subsidy to the water conservancy equipments.

4.2 The mechanism of circulating and making over the water rights in the Irrigation Area is established.

The established core of the water tights market is setting up the market of making over the water rights, i.e. on the foundation of the government' authorization, the independently operating and making over the water rights is permitted, that the households engaged in specialized water expenditure who didn't own the water rights or the quota is insufficient can acquire the water expenditure in agriculture is permitted. That circulating and making over the water tights can increase the efficiency of resources allocation. Once there is no valid circulating mechanism, water rights will remain and solidify in the current owners for long times, if things go on like this, then the phenomenon that low-efficiency in water expenditure will take place. Circulating and making over the water tights can handle and utilized by the farmer who is good at operation and can use the water in high efficiency? It can guarantee the water resources in high efficiency allocation and valid utilization throughout. The circulation means it can sell water outwardly., also can make over among the farmers in the Irrigation area.

4.2.1 The exterior circulation of the water rights in the Irrigation Area

From the above narration, selling water outwardly can increase the farmers' economic benefits. However, the actual business of selling water cannot do by the scatter farmers, and is done by the households collective. For the sake of selling water, the prior condition is to request the approval if every member, insisting the consistency approval principle. Namely, all the farmers must have the request of selling water. Hence, it's necessary to establish the farmers' water Expenditure Association. Extensive farmers' participation and publish of extensive information can lower the bargain cost and the management expenses. In additional, the farmers' opinions on selling water influence the system of water fee collecting. The exterior circulation should notice the below problems:

① On the calculation method of collecting the water fee, the following calculating formula is suggested:

The water price per stere ={ (the development expenses on water conservancy the same year) + (the maintenance and management expenses on water conservancy equipments)+ (the waster of electricity and thermal energy expenses)} / (the total water consumption of the irrigation Area).

② Selling the water outwardly should sell and collect the fee natively. The members cannot operate the business alone.

③ The income of selling water is allotted by the contribution degree of the composition of each member in this area. Here, each member' contributed amount on water selling is equal to his decreased water consumption of crops. The income of selling water is regard as allotted reward. Each member' reward = (the selling water' price per stare) × (the contributed amount of water selling).

④ The irrigation area does Water selling unify. Each member (farmer) cannot buy internally at lower price, then sell outwardly at higher price.

⑤ The valley management organization has the power to practice the mechanism of supervision and management in the magistracy.

4.2.2 The internal circulation of the water rights in the Irrigation Area

The internal circulation is among the different farmers. In a same irrigation area, the owned water consumption of the farmers is stable and the position is fixed relatively. The consultation and designed management facing the lower bargain cost than the exterior circulation. The internal circulation only needs the relevant parties' consultation, and examined to pass by the Water Expenditure Association.

4.3 To establish an intact responsibility, power, benefits system that do favor the management in the irrigation area.

During the two years of the management system reformation Changgang Irrigation Area probed continuously. The system construction pursued creation at every step. Only in the 2 years, 12 terms responsibility systems, 26 terms management systems, 9 terms contract-manage regulations, and 3 terms statutes are established. The establishment of these standardized system, made the whole irrigation area always keep neat in management. The farmers participate in the irrigative management positively. It mobilizes everyone' positivistic and creativity fully.

5. The evaluation on economic results

5.1 The efficiency in water use is increased, and the results is remarkable

5.1.1 The consciousness on water saving of the farmers increased relatively. The farmers participated in the management on expenditure, what enhanced their economy consciousnesses. They voluntarily adjusted the industrial structure, neated the actions lowed the water expenditure quota, reduced the loss from digging the ditch and the expenditure quota per 0.067 lectern from 800 stere to 500 stere since the establishment of the Water Expenditure Association.

5.1.2 Obey the principle that occupying water rights " first in time, first in right". i.e. to guarantee the rights of the occupier whose expenditure time is the longest isn't seized by the new occupier, to avoid the occurrence of the "pump-water contest" (In 1970s, the water rights wasn't definite in Los Angeles U.S.A. The occupying principle on land dominated the ownership to the under-ground water. The adjacent households competed in pumping water, even excessively used and wasted.), especially in the internal well-irrigation areas. Once the water rights aren't definite, the entry isn't limited. The occupying

principle dominated the ownership of the storage. The pumper owned the exclusive rights with the under-ground water to the others. At a pumper, the others now would pump the water he doesn't pump, and he can't occupy the water in the future if he doesn't take actions. Hence, the anxiousness impairing the pumper' motive that to decrease pumping water for the future." Pump-water contest " occurred inevitably.

5.1.3 It avoided the phenomenon that the village officer' hitchhike and charge much more, reducing the farmers' supports. That individuality provided money to buy constructive material and fixed the engineering is appeared. After the establishment of association, with the supervision system, the charging is transparent, the finance is public and expenditure is public, what lowed the cost.

5.1.4 The coefficient of ditch water usage also increased consumedly. It enhanced the engineering management in the irrigation area, transforming " public product" to "preparative public product". The branch ditches are managed and maintained by the group, and several households manage the small ditches. Before the establishment of the association, the water conservancy engineering is developed by the national investment. Once it was broken or in bad, the management unit does the maintenance. Hence, the farmers were totally unconcerned the maintenance on engineering. And the technique measures on water saving can't put into practice. After the establishment of the association, the quantity of ditches increased obviously. The engineering management has the link with their interests. Divided-segment management is practiced, and the duty is implemented. The farmers are positive to participate the management. The coefficient of ditch water usage also increased consumedly.

5.1.5 It strengthened the democracy management and democracy decision of the farmers, and increased the openness of the water expenditure management. Nowadays, the affairs must be passed by democracy negotiation and democracy decision. The principle of fair, reasonableness, and efficiency is emphasized. All kinds of water matters disputation were declined. 95% persons reflected that, since the establishment of the Water Expenditure Association. The disputations between the villages, the farmers, the households and the irrigation station reduced. The phenomenon that breaking the ditches, taking the soil in confusion, and digging the opening disorderly was eradicated.

5.1.6 It saved the main physical labors of the families, and gave full play to the women. He results of water saving is remarkable. Before the establishment of the association, in general, the males engaged in the guarding and pouring water. In the process of the association, there is no need to contest. The water expenditure quota, irrigative methods and the orders determine the irrigation. It's equal and fair. The women and the elder can participate is irrigation, which increased the achievement of the inferiority community. at the same time, the women are patience and meticulous in guarding water. The results of water saving is more remarkable. According to an inquisition, the males of 18 households guarded water formerly. Since the women managed the farmland, the average rate of water saving is above 10%.

5.1.7 The farmers hand in the water fee initiatively. Once the association funds is missing, the prepaid is allowed, which can make up the shortage of the funds.

5.2 The problems in solution

5.2.1 The benefits of the farmers is loss. The established lands of the public equipments, not attend by the form of partnership, but compensated once for all at a low price. That's unfair to the farmers for their long-term usage rights on the farmland. To let the farmers get advantage and long-term income by modern construction, it must allow the farmers join the partnership by making over the lands and have long-term bonus.

5.2.2 The function scope of the Water Expenditure Association is too narrow. It should extend to other community management or important activity. Such as the collectively relief when heavy natural disaster, fix the disaster-reducing engineering collecting, an-drought etc. The Water Expenditure Association should expend its function. Especially the effective water matter activities, it should have a hand in it.

5.2.3 The positive of feminine farmland management should be transferred further. The practice has proved that, on the management of the

irrigation system, the feminine position seems to be more important. The women who engaged in the farmland management, at the consciousness and methods on water saving and the careful degree, have advantage compared with the man. Also, that the women participate in the management, embodied two sexes' equality. And the efficiency of water saving and water expenditure is increased consumedly.

5.2.4 Sometimes thee management of the Water Expenditure Association conflicted with the local government, what made the in dilemma. On one hand, the farmers' supports is still overweight .The government personnel are excessive. The small agriculture economy can't support the huge superstructure. On the other hand, the power of county government is excessive, especially on the land-controlled power, what can seep into the private things. It caused the dissymmetry between the excessive power and the narrow sources of the revenue. The relations between the farmers and the officers is the relation of bringing up and be driven bringing up, not the relations that be served and service.

5.2.5 In the property right system reformation of the irrigation area, it must lay stress the handle of three relations. One is the relation between the national property keeping the value and the transforming of property right. (To definite the ownership, to make over the operation right, to definite the mature disposition right on the invested property of the operation personnel, to definite the redistribution of the property benefits which invested by the nation) Anther is the relation between the renter pursuing profits and the farmers' supports. And the last is the interests' relations between the renter and the workers of the irrigation area.

6 Conclusions

At present, the water rights reformation in Changgang Irrigation Area is still placed in initial stage. Lots of problems are treating perfect and deepen .In a short period; making over short-term water rights can be for lord. The essential point is to cultivate the yearly circulation bargain in different farmers of the same irrigation area. For short-term bargain is simply and easily organized .The long-term circulation, especially the exterior circulation, directly the long-term invested on the water conservancy. Therefore, it's necessary to cultivate the operation company on water conservancy equipments.

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Research on the Storage Methods of Lactic Acid Bacteria Strains Separated from DVS

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Abstract: Randomly amplified polymorphic DNA (RAPD) and morphological observation and fermentation property analysis were used to evaluate the different storage methods of three lactic acid bacteria strains separated from Directed Vat Set (DVS). **Methods:** Evaluate the genome change of the different samples from different periods during 4℃ storage, –80℃ storage and lyophilization using RAPD. Observe the morphological features by microscopy after recovered from different time of storage, then used each strain as a starter culture of fresh milk, observe the fermentation properties. **Results:** The RAPD patterns and fermentation property of the three strains all changed during 4℃ and –80℃ storage, and they all showed a good stability when stored by lyophilization. **Suggests:** This is the first time to use RAPD technique and fermentation property observation in lactic acid bacteria storage research work, RAPD appears to be an efficient method for evaluating of storage effect of lactic acid bacteria strains separated from DVS. [Nature and Science. 2004;2(4):49-53].

Keywords: DVS; lactic acid bacteria strains; storage method; fermentation properties; RAPD; morphological observation

1. Introduction

Lactic acid bacteria (LAB) are utilized in the production and preservation of various fermented foods (Jqrgen, 1999). Examples of such foods include yogurt, fermented milk, cheeses, fermented rice cake (puto), and various other foods of both plant and animal origin. Directed Vat Set (DVS) is a rapid, safety starter for the milk fermentation industry. But nobody knows what storage method is best for the LAB strains separated from DVS. The main aim of the present study was then to compare the storage methods of the LAB separated from DVS. Sonali Dixit, et al evaluated the genetic stability of plants regenerated from cryopreserved embryogenic tissues of *Dioscorea bulbifera* L. using RAPD. Finally the morphology and RAPD profiles all proved lyophilization is a proper storage method for the *Dioscorea bulbifera* embryogenic tissues (Sonali Dixit, 2003). This is the first time to compare different storage methods for the LAB strains separated from DVS using RAPD (Singh, 2004).

2. Materials and Methods

2.1 Strains: A *Streptococcus thermophillus* and two *Lactobacillus* which were separated from a DVS.

2.2 Separated Medium: M17 and MRS (Sandra, 1999) were used to separate the three strains.

2.3 Sugar Fermentation Experiment: To value the biochemical features of the three LAB strains by sugar fermentation experiment after separation. 12 different sugars were used in the experiment.

2.4 Storage Medium: The improved PY medium was used to stored in 4℃. 15% glycerin was added to the medium before it frozen in －80℃. Two improved silk milk media were used for *Streptococcus thermophillus*, *Lactobacillus* 1 and *Lactobacillus 2* lyophilization.

2.5 Morphological Features and Fermentation Properties During Storage: The strains were recovered respectively when they were stored for 1, 5, 10, 15, 30 d in 4℃. The same work was done when the strains were stored for 1, 15, 30 d in －80℃. The strains were recovered when they were stored for 90 d by lyophilization. The recovering time was recorded respectively (when the cells of the bacteria reached 10^8/ml), and the morphological features were

observed by microscopy. Each of the three LAB strains was used as the starter culture of fresh milk respectively (there were five equals for each strain), the average time was recorded when the milk changed into solid, then the average pH and qualities of the products were observed, thus the fermentation property were evaluated. All above features of the three LAB strains when just separated were tested as control.

2.6 RAPD Analysis During Storage: The three LAB strains at different time of storage were recovered respectively, then extracted their whole genome by CTAB(Kim,1990) as samples for RAPD.

PCR program was as follows: an initial denaturation consisting of 2 min at 94℃, 35 cycles consisting of 45 s at 94℃, 30 s at 38℃, and 30 s at 72℃, and then a final extension at 72℃ for 10 min. The PCR products were electrophoresed in 1.0% agarose gels, stained with ethidium bromide, and photographed.

3. Results

3.1 Sugar Fermentation Experiment (Table 1).

The sugar fermentation experiment of bacteria is shown in Table 1.

Table 1. The sugar fermentation experiment of bacteria

	Streptococcus thermophillus	*Lactobacillus 1*	*Lactobacillus 2*
Lactose	＋	＋	＋
D-Fructose	＋	＋	＋
D(+)Xylose	－	＋	＋
Sucrose	＋	－	＋
Mannitol	－	－	－
D-Sorbitol	－	－	－
Maltose	－	＋	＋
Glucose	＋	＋	＋
α , α -Trehalose	－	＋	＋
Esculin	＋	＋	＋
D-Galactose	＋	＋	＋
D-(+)-Cellobiose	＋	－	＋

'+' represented positive; '－' represented negative.

3.2 Morphological Features and Fermentation Properties During Storage

3.2.1 Morphological Features and Fermentation Properties of *Streptococcus thermophillus* During Storage (Table 2).

The Morphological features and fermentation properties of *Streptococcus thermophillus* during storage is shown in Table 2.

Table 2. Morphological features and fermentation properties of *Streptococcus thermophillus* during storage

Storage time (d)		Recovering time (h)	Morphological features	Fermentation Properties		
				Milk solidified time (h)	pH	Sense
4℃ storage	**1**	18	Cocci, singly and in short chain	5.5	4.72	Sour, milk flavor, taste common
	5	20	Cocci, in chain	6.5	4.73	Sour, not in good flavor
	10	24	Cocci, in chain	8	4.71	Sour, taste not good
	15	24	Cocci, in chain	12	4.71	Sour, taste not good
	30	24	Cocci, in chain	16	4.76	Sour, taste bad
–80℃ storage	**1**	18	Cocci, singly and in short chain	5	4.74	Sour, milk flavor, taste common
	15	24	Cocci, in chain	5.5	4.75	Sour, not in good flavor
	30	24	Cocci, in chain	8	4.73	Sour, taste not good
lyophilization	**90**	10	Cocci, singly and most in short chain	5	4.75	Sour, good flavor, taste best
Control			Cocci, in singly, pairs and short chain	5	4.73	Sour, good flavor, taste best

3.2.2 Morphological Features and Fermentation Properties of *Lactobacillus 1* During Storage (Table 3).

Morphological Features and Fermentation Properties of *Lactobacillus 1* During Storage is shown in Table 3.

Table 3. Morphological features and fermentation properties of *Lactobacillus 1* during storage

Storage time (d)		Recovering time (h)	Morphological features	Fermentation Properties		
				Milk solidified time (h)	pH	Sense
4℃ storage	1	24	Long rod, some in chain	13	4.71	Sour, flavor like milk
	5	28	Long rod, some in chain	15	4.73	Sour, not good flavor
	10	36	Long rod, in singly and chain	16	4.74	Not sour, taste bad
	15	48	Long rod, in singly and chain	18	4.76	Not sour, taste bad
	30	*				
–80℃ storage	1	24	Long rod, in singly and short chain	12.5	4.74	Sour, taste common
	15	26	Long rod, in singly and short chain	14	4.77	Sour, no good flavor
	30	26	Long rod, most in chain	16	4.76	Not sour enough
lyophilization	90	10	Long and thin rod, most singly	12.5	4.71	Sour, taste common
Control			Long and thin rod, in singly and short chain	12	4.70	Sour, taste common

**Lactobacillus 1* could not be recovered when it was store for 30 d in 4℃

3.2.3 Morphological Features and Fermentation Properties of *Lactobacillus 2* During Storage (Table 4).

The morphological Features and Fermentation Properties of *Lactobacillus 2* During Storage is shown in Table 4.

Table 4. Morphological features and fermentation properties of *Lactobacillus 2* during storage

Storage time (d)		Recovering time (h)	Morphological features	Fermentation Properties		
				Milk solidified time (h)	pH	Quality
4℃ storage,	1	24	Short rod, singly and in short chain	14	4.72	Not sour, good flavor
	5	30	Short rod, singly and in chain	15.5	4.76	Not sour, taste common
	10	38	Short rod, in short chain	16	4.74	Not sour, taste not good
	15	48	Short rod, most in chain	20	4.77	Not sour ,taste not good
	30	*				
–80℃ storage	1	24	Small rod, singly and in chain	13.5	4.72	Not sour, taste common
	15	30	Small rod, singly and in chain	14	4.73	Not sour, taste like milk
	30	30	Small rod, singly and in chain	16	4.72	Taste not good
Lyophilization	90	12	Small rod, most in chain, less singly	13	4.73	Not sour, good flavor
Control			Small rod, most in chain, some singly	13	4.72	Not sour, good flavor

**Lactobacillus 2* could not be recovered when it was store for 30d in 4℃.

3.3 RAPD Analysis When the Strains Were Stored in 4℃

The RAPD fingerprint of *Streptococcus thermophillus* changed a little since 5 d during 4℃ storage compared with 1 d (Figure 1, 1). The RAPD fingerprint of *Lactobacillus 1* changed a lot when stored for 10 d in 4℃ (Figure 1, 2). The RAPD fingerprint of *Lactobacillus 2* changed a lot from the 15 d (Figure 1, 3). Both the two *Lactobacillus* could not be recovered when they were stored for 30 d in 4 ℃.

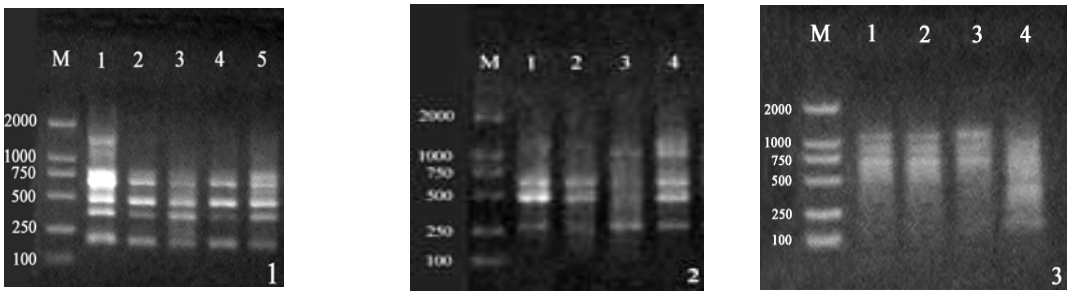

Figure 1. RAPD analysis of the strains stored for different time in 4℃. The bands were marker, stored for 1 d, stored for 5 d, stored for 10 d, stored for 15 d, stored for 30 d in proper order from left to right. (1) *Streptococcus thermophillus*. (2) *Lactobacillus 1*. (3) *Lactobacillus 2*.

3.4 RAPD Analysis When the Strains Were Stored in －80℃

The RAPD fingerprint of *Streptococcus thermophillus* changed since 15 d during －80℃ storage (Figure 2, 1). The RAPD fingerprint of *Lactobacillus 1* changed a lot when stored for 15 d, no pattern produced when stored for 30 d (Figure 2, 2). The RAPD fingerprint of *Lactobacillus 2* changed a lot during －80 ℃ storage, and no pattern produced when stored for 30 d (Figure 2, 3).

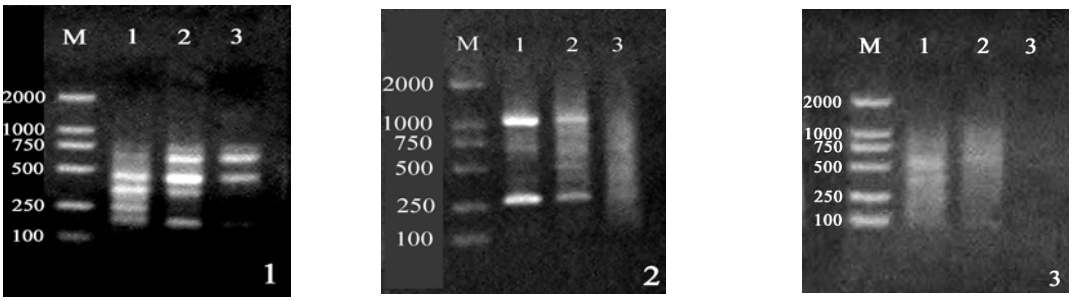

Figure 2. RAPD analysis of the strains stored for different time in －80℃. The bands were marker, stored for 1 d, stored for 15 d, stored for 30 d in proper order from left to right. (1) *Streptococcus thermophillus*. (2) *Lactobacillus 1*. (3) *Lactobacillus 2*.

3.5 RAPD Analysis When the Strains Were Stored by lyophilization

The RAPD fingerprint of the three strains did not change when they were stored by lyophilization (Figure 3).

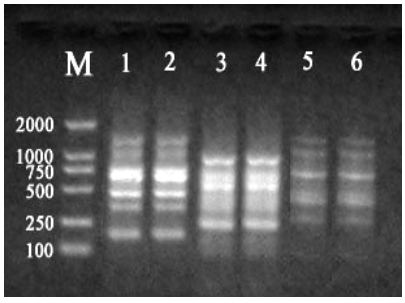

Figure 3. RAPD analysis of the strains stored by lyophilization. The bands were marker, *Streptococcus thermophillus* before lyophilization, *Streptococcus thermophillus* after lyophilization, *Lactobacillus 1* before lyophilization, *Lactobacillus 1* after lyophilization, *Lactobacillus 2* before lyophilization, *Lactobacillus 2* after lyophilization in proper order from left to right.

4. Discussion

It is known that some properties of bacteria may change when they are stored. The result of RAPD and observation of morphology and fermentation properties showed that 4℃ and －80℃ were unfit for the three strains' long time storage, after 5 days storage the fermentation properties got worse and RAPD fingerprint changed too. However, morphological features and fermentation property and RAPD fingerprint of the three LAB strains didn't change obviously after 90 d store by lyophilization.

RAPD is an easy and cost-effective profiling assay based on PCR with arbitrary primers that, by amplifying a set of DNA segments randomly distributed throughout the genome, can detect genetic polymorphisms. Nevertheless, RAPD techniques have some limitations concerning reproducibility and an uncertain homology of co-migrating fragments in gel electrophoresis. But most of these limitations can be minimized by carefully adjusting the reaction and detection conditions (Lusia C. Carvalho, 2004). In our work, we found that the RAPD fingerprint of the control group (three LAB strains) all are of certainty and reproducible.

Though LAB is wide spread in the natural environment and play an important role in several industrial and food fermentations, it is difficult to store them without function changes of these strains. So a perfect way to store LAB strains is very important to research a good starter culture for fermentation industry. The present study shows that lyophilization is the best choice for LAB strains storage through morphology observation and fermentation analysis and RAPD analysis. This is the first time to use RAPD technique as a tool to analysis LAB strains' property change when they are stored.

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Agricultural Mechanization Contribution Rate Calculation Software Package Development

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Abstract: The main purpose that calculates the contribution rate of mechanization in agriculture to the development of it is to recognize the real function of mechanization to the increasing output and income. In practice the calculation is good to know the development level, potentialities and trend of mechanization in agriculture from overall. It also offers the scientific base for decision making department to instruct and promote work of mechanization in agriculture. This paper created software specially to calculate the contribution rate of mechanization in agriculture by using economy math method, computer technology and Visual Basic 6.0 version. The software package has friendly interface, simply operating way and accurately, feasibly calculating method. It greatly changed the condition in the past which has considerable amount of data to be dealt, miscellaneous and trivial methods, and hard to seek answer, and it has very high practical value. [Nature and Science. 2004;2(4): 54-56].

Key Words: mechanization in agriculture; rate of contribution; calculation; software package

1 System function design

The main function of the software is to calculate the contribution rate of mechanization in agriculture with five different methods, save its results and maintenance the system. The five methods are Solow Complementary Value Method, Cobb-Douglass Production Function Method, Data Envelopment Analysis Method, Delphi Method and Pluralistic Linear Regression Method. We studied two different instances in Solow Complementary Value Method. One side the coefficient of speed-increasing equation is constant, and the other side the coefficient is variable with time(Wang, 1996) The system flow chart of the software is showed as Figure 1.

2. System interface design

Interface design of the software is designed relying on form by using Object Oriented Programming idea. All system forms have identical style. Some interface design content will be expatiated such as identity attestation interface, main control interface and input and output interface (Evangelos, 1999; Zong, 2000).

2.1 Identity attestation interface design

An identity attestation forms is designed for system security preventint unlawful user from using the system. You must load first when you use the syetem every time. The system also offers a modified function for loading information. The user loading interface and modified logging information interface are showed as Figure 2 and Figure 3.

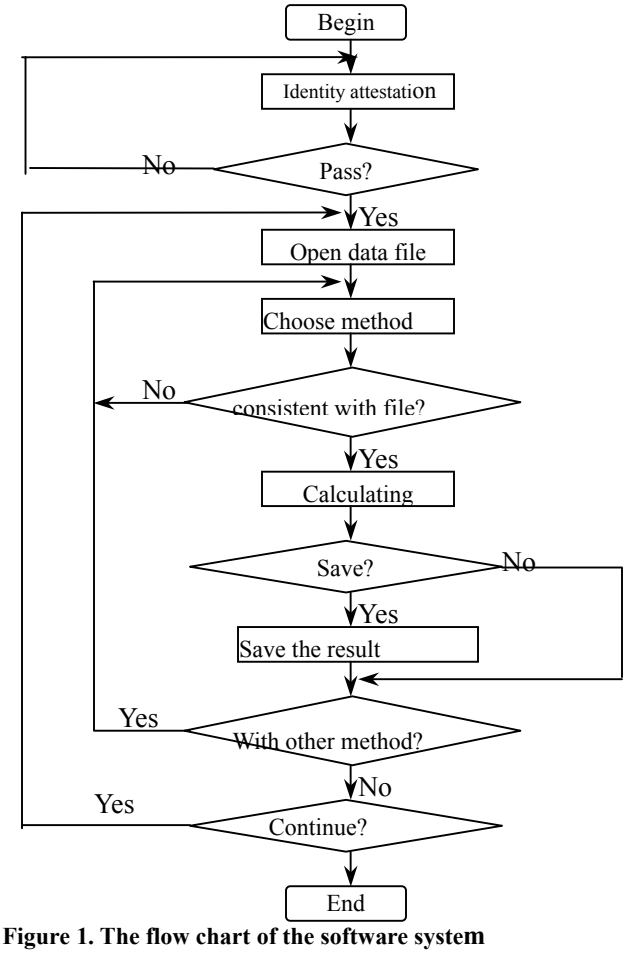

Figure 1. The flow chart of the software system

Figure 2. User logging interface

Figure 3. Modified logging information interface

2.2 Main control interface design

Main control interface design is the first step of system program design. It is connected with system holistic capability and function implement. Main control interface of the software include menu column, method selection area, function implement area and result output area. In method selection area there are five method buttons concluded. In function implement area conclude four command button such as run, close, clear screen and save. In which pressing run button can calculate the contribution rate of mechanization in agriculture, and pressing clear and save button can clear and save information of result output area. Main control interface is show as Figure 4.

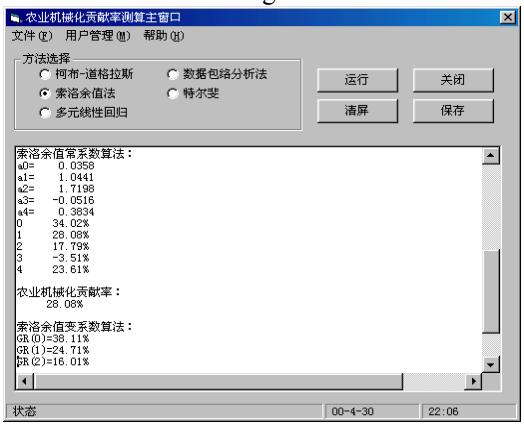

Figure 4. Main controlling interface of SCMA

2.3 Input and output interface design

Input and output interface design is a main part of system design. It often takes user much time. We will expatiate the design of input and output interface.

2.3.1 Input interface design

Input interface of the software is designed as interactive grid. Interface upside is a grid of input datum, and underside is parameter input area and command button area. For each sort of information there is an item to indicate the content and position. Otherwise input datum type can be tested automatically and user also can modify the datum.

In the system input datum can be divided into two parts. In which input datum of four methods such as Solow Complementary Value Method, Cobb-Douglass Production Function Method, Delphi Method and Pluralistic Linear Regression Method are the same kind. The file extensible name is defined .dt1. It has the same input interface showed as Figure 5. Input datum of Data Envelopment Analysis Method is the other kind. Its file extensible name is defined .dt2. Its input datum interface is showed as Figure 6.

Figure 5. Input interface of SOLOW Complementary Value Method

Figure 6. Input interface of Delphi Method

2.3.2 Output interface design

Output results of the system are showed underside of main control interface, which showed the parameters we calculated and some middle results. Output interface is showed as Figure 4.

3. Conclusion

The software package, which has friendly interface, simple operating way and accurate, feasible calculating method. It improves the working efficiency greatly. The calculation software offered the scientific base for decision making department to instruct and promote works of mechanization in agriculture on practice.

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Control of the Behavior Dynamics for Motion Planning of Mobile Robots

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Abstract: A new approach to the motion planning problems of mobile robots in uncertain dynamic environments based on the behavior dynamics is proposed. The fundamental behavior of a mobile robot in motion planning, which is regarded as a dynamic process of the interaction between the robot and its local environment, is modeled and controlled for the motion planning purpose. The behavior dynamics is the law in a dynamic process that the involved objects must obey, and it is controlled by the robot's dynamics. Based on the control of the behavior dynamics, the dynamic motion-planning problem of the mobile robots is transformed into a control problem of the integrated planning-and-control system. No restrictions are assumed on the shape and trajectories of obstacles. Collision avoidance between multiple mobile robots can also be realized. The stability of the integrated planning-and-control system can be easily guaranteed. Simulations illustrate our results. [Nature and Science. 2004;2(4):57-64].

Key Words: Behavior dynamics; motion planning; mobile robots; uncertain dynamic environment

1. Introduction

In all the applications of mobile robots, a good motion planning method is very important to accomplish tasks efficiently and stably [1-2]. However, whenever it comes to dealing with an environment that is totally or partially unknown or even dynamically changing, such a dynamic motion-planning problem is intractable [3], since the mobile robot is required to decide its motion behavior on line using only sensors' limited information. Various methods have been proposed for this purpose, such as (1) Configuration-time space based methods [4-7,30], (2) Planning in space and time independently [8-11], (3) Artificial potential fields based methods [12-18], (4) Behavior based methods [19-20], (5) Intelligent computing based methods [20-24]. Some new methods are also proposed recently, *e.g.*, cooperative collision avoidance and navigation [20,25,26], velocity obstacles method [27], collision cone approach [28], etc. Other sensor-based navigation frameworks can also refer to [13,29]. Significant improvements on the motion-planning problems of a robot have been obtained in the past decades. However, many of the existing methods are only kinematic planning, or rely on some knowledge of the global environment, or require some constraints on the shape or velocity of obstacles, etc. Moreover, behavior decision-making usually is to simply sum up all the impacts from different obstacles with different weights in conventional behavior-based methods [19]. This may lead to counteraction of different reactive behaviors, and consequently result in unexpected motion behavior or performance of the mobile robot.

In motion planning problems of mobile robots, motion behaviors of the mobile robot can be classified into two fundamental behaviors: Collision-Avoidance behavior, and Going-to-the-Goal behavior (in short, CA-behavior and GG-behavior, respectively). How these behaviors are realized and performed in motion planning, how the goodness of the motion planning is. In most of the existing motion-planning methods, motion behaviors are generally regarded as static and discrete behavioral reactions to the environments, instead of dynamic processes. These methods cannot effectively map the local changing environment into dynamic behaviors of the robot. The reactive behaviors, together with the robot dynamics, cannot be integrated into one uniform planning-and-control system to be designed to achieve some performances of the whole system. For these reasons, the conventional behavior-based methods may offer poor performance for the robotic system of large mass or high velocity.

In this paper, the fundamental behaviors of a

mobile robot is modeled as dynamic processes of the interactions between the robot and its local environments, then the desired dynamic motion behaviors can be obtained based on the control of these dynamic processes, which are called behavior dynamics. The control input of the behavior dynamics is right the desired acceleration of the mobile robot. The behavior dynamics and the robot's dynamics are integrated into one uniform planning-and-control system, and thus a new method using only sensors' information for the motion planning of mobile robots in uncertain dynamic environments is proposed. The behavior dynamics is sensor-based, and no knowledge of the shape and velocity of the obstacles are needed. The dynamic constraints of the mobile robot are considered. Collision avoidance between multiple mobile robots can also be realized. The stability of the whole planning-and-control system can be guaranteed. To the best of our knowledge, this behavior dynamics-based approach is novel. Simulations are given to illustrate our method.

Notations: A black bold symbol denotes a vector, *e.g.* a vector $\boldsymbol{V}$, and then V denotes its norm. $x = \arg(\cdot)$ means to choose an x satisfying $(\cdot)$. For any vector $\boldsymbol{A}$, let $\boldsymbol{e}(\boldsymbol{A}) = \boldsymbol{A} / \|\boldsymbol{A}\|$. A point p in a plane is written as $\boldsymbol{p}=(p_x, p_y)$, or $\boldsymbol{p} = [p_x, p_y]^T$, where p_x and p_y are the corresponding components on each coordinate. Let $\boldsymbol{e}_X = [1,0]^T$.

2. Modeling of the fundamental behaviors in motion planning

In this section, behavior dynamics is defined and modeled. Without loss of generality, we regard a mobile robot as a point mass, and restrict our study to the 2-D planar case. Similar method can be extended to more than 2-D cases.

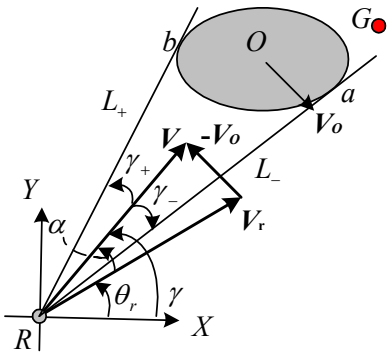

Figure 1. The robot meets an obstacle

At time t, without speciality, refer to Figure 1. The mobile robot $\boldsymbol{R}$ is at the original point of the global coordinates $\{X,Y\}$ with velocity $\boldsymbol{V_r}$ and meets an obstacle at point $\boldsymbol{O}$ with velocity $\boldsymbol{V_O}$,. Let point $\boldsymbol{G}=(x_d,y_d)$ be the goal of the robot, and $\boldsymbol{V}=\boldsymbol{V_r}-\boldsymbol{V_O}$, be the relative velocity of the mobile robot with respect to the obstacle. The angle from vector $\boldsymbol{V_r}$ and $\boldsymbol{V_O}$ to vector $\boldsymbol{V}$ are denoted by α and β, which are also written as $\alpha = \angle(\boldsymbol{V}, \boldsymbol{V_r})$ and $\beta = \angle(\boldsymbol{V}, \boldsymbol{V_O})$, respectively. This paper assumes that, all the angles in this paper belongs to $[-\pi, \pi]$, and an angle is positive if it is formed by rotating a vector anti-clockwise, else it is negative. In Figure 1, $\gamma, \theta_r, \theta_O$ are the angles from X-coordinate to the vectors $\boldsymbol{V}$, $\boldsymbol{V_r}$, and $\boldsymbol{V_O}$, respectively. Obviously, $\gamma = \theta_r + \alpha = \theta_O + \beta$. From point R, there are two lines that are tangent to the boundary of the obstacle O and denoted by L_+ and L_-. The angles from vector $\boldsymbol{V}$ to L_+ and L_- are denoted by γ_+ and γ_-, respectively. For any vector $\boldsymbol{V} \neq 0$, it cannot only be written as $\boldsymbol{V} = [V_x, V_y]^T$ using its corresponding components on each coordinate, also be denoted by $[V, \gamma]^T$, where $V = \|\boldsymbol{V}\|$, $\gamma = \angle(\boldsymbol{V}, \boldsymbol{e_X})$. For the two forms, we have

$$V_x = V\cos\gamma, \text{and } V_y = V\sin\gamma. \tag{1}$$

Using the variables given above, the collision condition can be written as

$$\gamma_+ \cdot \gamma_- \leq 0 \tag{2}$$

Once inequality (2) holds, the mobile robot is heading a collision. That is, if inequality (2) holds, the relative velocity vector $\boldsymbol{V}$ should be controlled to rotate a desired angle γ_+ or γ_- in order to avoid a collision. This is the dynamic process of a collision-avoidance behavior. The model of this dynamic process is developed in the following.

From (1), it is easy to obtain the following relationship:

$$\dot{\boldsymbol{V}} = \begin{bmatrix} \dot{V}_x \\ \dot{V}_y \end{bmatrix} = \boldsymbol{J}(V,\gamma) \cdot \begin{bmatrix} \dot{V} \\ \dot{\gamma} \end{bmatrix} \text{ or}$$

$$\begin{bmatrix} \dot{V} \\ \dot{\gamma} \end{bmatrix} = \boldsymbol{J}(V,\gamma)^{-1} \cdot \begin{bmatrix} \dot{V}_x \\ \dot{V}_y \end{bmatrix} \tag{3}$$

where $\boldsymbol{J}(V,\gamma) = \begin{bmatrix} \cos\gamma & -V\sin\gamma \\ \sin\gamma & V\cos\gamma \end{bmatrix}$.

Recalling that $\boldsymbol{V}=\boldsymbol{V_r}-\boldsymbol{V_O}$, and from (3) we have

$$\begin{bmatrix} \dot{V} \\ \dot{\gamma} \end{bmatrix} = \boldsymbol{J}(V,\gamma)^{-1} \cdot \begin{bmatrix} \dot{V}_x \\ \dot{V}_y \end{bmatrix} = \boldsymbol{J}(V,\gamma)^{-1} \cdot \left(\begin{bmatrix} \dot{V}_{rx} \\ \dot{V}_{ry} \end{bmatrix} - \begin{bmatrix} \dot{V}_{Ox} \\ \dot{V}_{Oy} \end{bmatrix} \right) \tag{4}$$

Utilizing (3a), Equation (4) yields

$$\begin{bmatrix}\dot{V}\\ \dot{\gamma}\end{bmatrix} = \boldsymbol{J}(V,\gamma)^{-1} \cdot \left(\boldsymbol{J}(V_r,\theta_r)\cdot\begin{bmatrix}\dot{V}_r\\ \dot{\theta}_r\end{bmatrix} - \boldsymbol{J}(V_O,\theta_O)\cdot\begin{bmatrix}\dot{V}_O\\ \dot{\theta}_O\end{bmatrix}\right) \quad (5)$$

Recalling that $\gamma = \theta_r + \alpha = \theta_O + \beta$, and the following relationships

$$\left.\begin{aligned}\sin(\omega+\sigma) &= \sin\omega\cdot\cos\sigma + \cos\omega\cdot\sin\sigma\\ \cos(\omega+\sigma) &= \cos\omega\cdot\cos\sigma - \sin\omega\cdot\sin\sigma\end{aligned}\right\} \quad \forall\omega,\sigma$$ with some calculations, Equation (5) further yields

$$\begin{bmatrix}\dot{V}\\ \dot{\gamma}\end{bmatrix} = \begin{bmatrix}\cos\alpha & V_r\sin\alpha\\ -\frac{1}{V}\sin\alpha & \frac{V_r}{V}\cos\alpha\end{bmatrix}\cdot\begin{bmatrix}\dot{V}_r\\ \dot{\theta}_r\end{bmatrix} - \begin{bmatrix}\cos\beta & V_O\sin\beta\\ -\frac{1}{V}\sin\beta & \frac{V_O}{V}\cos\beta\end{bmatrix}\cdot\begin{bmatrix}\dot{V}_O\\ \dot{\theta}_O\end{bmatrix} \quad (6)$$

Equation (6) is the Behavior Dynamics of a Collision-Avoidance behavior, in short as CA-dynamics. It is the law that the involved robot and obstacle must obey in a collision- avoidance process. Equation (6) is developed with assumption that $\boldsymbol{V} \neq 0$. It is noted that $\boldsymbol{V}$ =0 can not hold for all the time in order to avoid an obstacle, thus this paper only considers the case $\boldsymbol{V} \neq 0$. In each planning period, $\boldsymbol{V_O}$ can be assumed to be constant. Hence, we have $\dot{V}_O = 0$ and $\dot{\theta}_O = 0$. Otherwise, the last term of equation (6) can be estimated and thus its effect can be cancelled using some methods. Then (6) can be rewritten as follows:

$$\begin{bmatrix}\dot{V}\\ \dot{\gamma}\end{bmatrix} = \begin{bmatrix}\cos\alpha & V_r\sin\alpha\\ -\frac{1}{V}\sin\alpha & \frac{V_r}{V}\cos\alpha\end{bmatrix}\cdot\begin{bmatrix}\dot{V}_r\\ \dot{\theta}_r\end{bmatrix} \quad (7a)$$

As for the CA-dynamics in (7a), the control input is $[\dot{V}_r, \dot{\theta}_r]^T$, it is easy to be transformed into $[\dot{V}_{rx}, \dot{V}_{ry}]^T$, which is the acceleration of the mobile robot in the global coordinates. It should be noted that α , V and γ can all be detected or calculated in the local coordinates on the mobile robot, therefore, there are only sensor information needed in Equation (7a). This facilitates the motion planning for mobile robots in dynamic uncertain environments. Once Inequality (1) holds, the control problem of (7a) is: *Given the desired* $[V_d, \gamma_d]^T$ *for a collision-avoidance behavior, the task is to find a control law*

$$\begin{cases}\dot{V}_r = u_V(V_d, V, \gamma_d, \gamma, \alpha)\\ \dot{\theta}_r = u_\theta(V_d, V, \gamma_d, \gamma, \alpha)\end{cases} \quad (7b)$$

such that system (7) is stable, i.e.,

$$\lim_{t\to T_1} V(t) = V_d, \quad \lim_{t\to T_1}\gamma(t) = \gamma_d \qquad (\exists T_1 > 0).$$

Though $[V_d, \gamma_d]^T$ is time varying, it can be regarded as constant in each planning period. For a collision-avoidance behavior, such that $\gamma_+ \cdot \gamma_- < 0$ does not hold, should only γ_d be set to be γ_+ or γ_- for the above control problem whatever V is. That is, γ is the key variable to be controlled in system (7) to avoid an obstacle, and V_d can be set to be any positive value. For this reason, as for the CA-dynamics we need only consider a simplified system as follows:

$$\dot{\gamma} = \begin{bmatrix}-\frac{1}{V}\sin\alpha & \frac{V_r}{V}\cos\alpha\end{bmatrix}\cdot\begin{bmatrix}\dot{V}_r\\ \dot{\theta}_r\end{bmatrix} \quad (8)$$

That is, only the steering angle of $\boldsymbol{V}$ is considered.

If the goal of the mobile robot is regarded as a special obstacle, then the CA-dynamics can also be regarded as the dynamics of GG-behavior. In this case, $\boldsymbol{V}=\boldsymbol{V_r}$, $\alpha = 0$. Then Equation (7a) is now written as

$$\begin{cases}\dot{V} = \dot{V}_r\\ \dot{\gamma} = \dot{\theta}_r\end{cases} \quad (9)$$

Obviously, (9) is included in system (7a), and $[V_d, \gamma_d]^T$ in (7b) for GG-behavior is to be defined.

Another behavior frequently generated in a collision-avoidance process or navigation of the mobile robot is the Wall Following (WF) behavior, which is to follow the boundary of an obstacle till avoiding it. The dynamics of WF-behavior is the same to (9), and the desired $[V_d, \gamma_d]^T$ in (7b) for WF-behavior is also to be defined. If there exists collision risk, then CA-behavior is carried out. Once there is no collision risk but the obstacle is still near enough to the mobile and between the mobile robot and its goal, then WF-behavior is generated in this case.

From above all, it is noted that all the fundamental behaviors in motion planning problem can be described by the same dynamics model (7) with different desired $[V_d, \gamma_d]^T$.

3. Control of the behavior dynamics and the robot dynamics

In order to illustrate our idea, only a simple case for the robot dynamics is considered in this paper. For other cases, similar results can be developed. Hence, for simplicity, the robot dynamics considered in this paper is described by

$$\boldsymbol{M(q)\ddot{q} + f(q,\dot{q})\dot{q} + g(q) = \tau} \quad (10)$$

Using the computed torque control

$$\boldsymbol{\tau = M(q)u + f(q,\dot{q})\dot{q} + g(q)} \quad (11)$$

then (10) can be rewritten as

$$\boldsymbol{\ddot{q} = u} \quad (12)$$

It is now needed to plan the desired states $[\ddot{\boldsymbol{q}}_{\boldsymbol{d}}^{\boldsymbol{T}}, \dot{\boldsymbol{q}}_{\boldsymbol{d}}^{\boldsymbol{T}}, \boldsymbol{q}_{\boldsymbol{d}}^{\boldsymbol{T}}]^T$ for (12). Utilizing (7b), we have

$$\begin{aligned} \ddot{\boldsymbol{q}} &= \begin{bmatrix} \dot{V}_x \\ \dot{V}_y \end{bmatrix} = \boldsymbol{J}(V_r, \theta_r) \cdot \begin{bmatrix} \dot{V}_r \\ \dot{\theta}_r \end{bmatrix} \\ &= \boldsymbol{J}(V_r, \theta_r) \cdot \begin{bmatrix} u_V(V_d, V, \gamma_d, \gamma, \alpha) \\ u_\theta(V_d, V, \gamma_d, \gamma, \alpha) \end{bmatrix} \end{aligned} \tag{13}$$

where, $V_r = \|\dot{\boldsymbol{q}}\|, \theta_r = \angle(\dot{\boldsymbol{q}}, \boldsymbol{e}_X)$. (13) is the desired $\ddot{\boldsymbol{q}}_{\boldsymbol{d}}$ for (12). This implies that it needs only to control the acceleration of the mobile robot for a desired behavior. Now, the integrated planning-and-control system based on behavior dynamics can be described as:

$$\begin{cases} \dot{\boldsymbol{v}} = \boldsymbol{B}(V, V_r, \alpha, \theta_r) \cdot \ddot{\boldsymbol{q}} & (14a) \\ \ddot{\boldsymbol{q}} = \boldsymbol{u} & (14b) \end{cases}$$

which further yields

$$\dot{\boldsymbol{v}} = \boldsymbol{B}(V, V_r, \alpha, \theta_r) \cdot \boldsymbol{u} \tag{15a}$$

where, $\boldsymbol{v} = \begin{bmatrix} V \\ \gamma \end{bmatrix}$,

$$\mathbf{B}(V, V_r, \alpha, \theta_r) = \begin{bmatrix} \cos\alpha & V_r \sin\alpha \\ -\frac{1}{V}\sin\alpha & \frac{V_r}{V}\cos\alpha \end{bmatrix} \cdot \mathbf{J}(V_r, \theta_r)^{-1}$$

From (14), control of the behavior dynamics is in fact a control problem in the acceleration space of the mobile robot. The control problem for (15a) is: *Given a desired* $\boldsymbol{v}_d = [V_d, \gamma_d]^T$ *for a desired behavior, to find the control law* $\boldsymbol{u}$*, such that* $\lim_{t\to\infty} \boldsymbol{v}(t) = \boldsymbol{v}_d$. For this problem, the following result is obvious.

Theorem 1. Given a desired $\boldsymbol{v}_d$ for (15a), choose any a simple linear feedback control law $\boldsymbol{u}$ as follows:

$$\boldsymbol{u} = -\boldsymbol{K}_1 \boldsymbol{e}_v \tag{15b}$$

Then (15a) is asymptotically stable, *i.e.*, $\lim_{t\to\infty} \boldsymbol{v}(t) = \boldsymbol{v}_d$. Where, $\boldsymbol{e}_v = \boldsymbol{v} - \boldsymbol{v}_d, \boldsymbol{B}\boldsymbol{K}_1 > 0$.

It should be noted that (15b) is the desired $\ddot{\boldsymbol{q}}_{\boldsymbol{d}}$ for the mobile robot. To realize a desired behavior, is to control the robot's dynamics to follow a desired acceleration. For this reason, we can let $\dot{\boldsymbol{q}}_{\boldsymbol{d}}(t) = \dot{\boldsymbol{q}}(t_0) + \int_{t_0}^{t} \ddot{\boldsymbol{q}}_{\boldsymbol{d}}(s)ds$, and

$\boldsymbol{q}_{\boldsymbol{d}}(t) = \boldsymbol{q}(t_0) + \int_{t_0}^{t} \dot{\boldsymbol{q}}_{\boldsymbol{d}}(s)ds$.

Then for the robotic system, it is easy to design a tracking control law to guarantee the realization of the desired behavior.

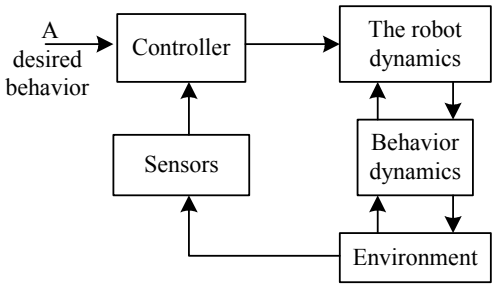

Figure 2. A structure of the whole system

From aforementioned discussions, stability of a desired behavior and the whole planning-and-control system is easy to be guaranteed. The motion-planning problem is now transformed into a control problem of the behavior dynamics. A structure of the whole system can be referred to Figure 2. By using behavior dynamics, the planning task is now to give the desired $\boldsymbol{v}_d$ at each planning time. Various methods can be adopted to plan the desired $\boldsymbol{v}_d$, a simple method is provided in the following section.

4. Realization of the desired behaviors

In this section, we design the desired $\boldsymbol{v}_d$ using the local knowledge of the environment in order to realize the desired behaviors for the mobile robot according to the control laws in Theorem 1.

As for the GG -behavior, let $[V_d, \gamma_d]^T$ in (7b) be:

$$\begin{cases} V_d = \min(0.7 \cdot V_{\max}, \sqrt{\|\boldsymbol{G} - \boldsymbol{R}\|}) \\ \gamma_d = \angle(\boldsymbol{e}(\boldsymbol{G} - \boldsymbol{R}), \boldsymbol{V}_r) \end{cases} \tag{16}$$

where, V_{max} is a positive constant, $\boldsymbol{G}=(x_d,y_d)^{\mathrm{T}}$ and R are the goal and current position of the mobile robot, $\boldsymbol{e}(\boldsymbol{G}\text{-}\boldsymbol{R})$ is the desired direction for the robot to move in the global coordinates, which is assumed to be known. And the desired $[V_d, \gamma_d]^T$ in (7b) for WF-behavior is given as

$$\begin{cases} V_d = \min(0.7 \cdot V_{\max}, \sqrt{\|\boldsymbol{G} - \boldsymbol{R}\|}) \\ \gamma_d = \arg\min_{\gamma \in \{\gamma_+, \gamma_-\}} (|\gamma|) \end{cases} \tag{17}$$

where $\{\gamma_+, \gamma_-\}$ corresponds to the obstacle to be followed.

Since the relative velocity is the robot's velocity for the GG/WF-behavior, we have

$$\dot{\boldsymbol{q}}_{\boldsymbol{d}} = \begin{bmatrix} V_d \cos\gamma_d \\ V_d \sin\gamma_d \end{bmatrix}, \text{ and } \ddot{\boldsymbol{q}}_{\boldsymbol{d}} \text{ is (17b), } \boldsymbol{q}_{\boldsymbol{d}}=[x_d,y_d]^{\mathrm{T}}. \tag{18}$$

where $V_d = \min(0.7 \cdot V_{\max}, \sqrt{\|\boldsymbol{G}-\boldsymbol{R}\|})$.

As for the CA-behavior, we only consider the control problem of the simplified CA-dynamics (8). If $\gamma_+ \cdot \gamma_- \le 0$, we let

$$\gamma_d = \arg\min_{\gamma\in\{\gamma_+,\gamma_-\}}(|\gamma|) \tag{19}$$

The $\ddot{\boldsymbol{q}}_{\boldsymbol{d}}$, $\dot{\boldsymbol{q}}_{\boldsymbol{d}}$ and $\boldsymbol{q}_{\boldsymbol{d}}$ are still (18).

Considering the dynamic constraints of the mobile robot, that is, the maximum velocity and acceleration are assumed to be V_{max} and a_{max}, respectively. Let $P(R, t)$ be the observable region of the mobile robot, in which an obstacle can be effectively detected by the sensors. For an obstacle O_i, let ∂D_i be its boundary. If there exists a point $P \in \partial D_i \cap P(R,t)$,such that $|\angle(\boldsymbol{e}(\boldsymbol{V}_i), \boldsymbol{P}-\boldsymbol{R})| = 0 \text{ or } \pi$,then let $\boldsymbol{P}_{ci} = \arg\left(|\angle(\boldsymbol{e}(\boldsymbol{V}_i), \boldsymbol{P}-\boldsymbol{R})|_{\boldsymbol{P}\in\partial D_i\cap P(\boldsymbol{R},t)} = 0 \text{ or } \pi\right)$, otherwise let $\boldsymbol{P}_{ci} = \infty$ .Let$\boldsymbol{V_i}=\boldsymbol{V_r}-\boldsymbol{V_{Oi}}$, and $V_i = \|\boldsymbol{V}_i\|$. Let $l_i = (\boldsymbol{P}_{ci}-\boldsymbol{R})\cdot\boldsymbol{e}(\boldsymbol{V}_i)$ be the collision distance (C-distance) of the mobile robot and an obstacle O_i. If the mobile robot is heading to an obstacle O_i and the distance l_i is too near, only going in the contrary direction with the maximum acceleration can the robot guarantee the safety. It is easy to prove that the minimum distance for this case is $l_0 = \frac{V_i^2}{2(a_{\max} + \dot{\boldsymbol{V}}_{O_i}\boldsymbol{e}(\boldsymbol{V}_i))}$. If the obstacle is static or its velocity is changing slowly, *i.e.*, $\dot{\boldsymbol{V}}_{\boldsymbol{O}_i} \approx 0$, then $l_0 = \frac{V_r^2}{2a_{\max}}$. Once the robot is approaching to this distance, the steering angle γ of the relative velocity $\boldsymbol{V_i}$ should be modified to the direction opposite to the current direction. For this purpose, we have the following results.

For an obstacle O_i, we redesign the γ_d in (19) to be

$$\begin{cases}\gamma_{ds} = \arg\min_{\gamma\in\{\gamma_+,\gamma_-\}}(|\gamma|)\\ \gamma_d = \gamma_{ds} + \frac{\pi - |\gamma_{ds}|}{1+k_s\cdot pos(l_i-l_0)}\cdot sign(\gamma_{ds})\end{cases} \tag{20}$$

where, $\forall x$, $pos(x)=\max(0,x)$,

$sign(x) = \begin{cases}1 & x\ge 0\\ -1 & x<0\end{cases}$, $k_s>0$. Note that $\gamma_d \to \pm\pi$ whenever $l_i \to l_0$, and $\gamma_d \to \pm\gamma_{ds}$ if $l_i = \infty$.

Note that (20) provides the γ_d only for one obstacle case. Considering the multiple obstacles case, we need give the optimal γ_d for the robot to go according to all the steering angles of the relative velocity $\boldsymbol{V_i}$ corresponding to different obstacles.

We have mentioned that there are two steering angles, *i.e.* γ_{i+} and γ_{i-} , corresponding to an obstacle O_i. And in order to avoid the obstacle, the relative velocity $\boldsymbol{V_i}$ should be adjusted such that $\gamma_{i-} \le \gamma_i \le \gamma_{i+}$ not holds. Let $D_i = \{\gamma \mid \gamma_{i-} \le \gamma \le \gamma_{i+}\}$, it is called dangerous region with respect to the obstacle O_i. Considering the idea used in (20), D_i can be rewritten as

$$D_i = \{\gamma \mid \gamma_{i-} + \delta(\gamma_{i-}) \le \gamma \le \gamma_{i+} + \delta(\gamma_{i-})\} \tag{21}$$

where, $\delta(\gamma) = \frac{\pi - |\gamma|}{1+k_s\cdot pos(l_i-l_0)}\cdot sign(\gamma)$. Then for all the obstacles in the observable region $P(R, t)$, the dangerous region is

$$D = \bigcup_i D_i \tag{22}$$

Then from (22), the decision-making space of γ_d for the mobile robot is

$$U = [-\pi,\pi] \setminus (\bigcup_i D_i) \tag{23}$$

The desired γ_d for the robot to avoid the obstacles should be chosen from U. However, a constraint for this decision-making corresponding to the dynamic constraints of the mobile robot should be satisfied. From (15b) and note that $\|\ddot{\boldsymbol{q}}_{\boldsymbol{d}}\| \le a_{\max}$, we have $\|\boldsymbol{K}_1\gamma_d\| \le a_{\max}$. Hence, we have $\gamma_d \le \|\boldsymbol{K}_1\|^{-1} a_{\max}$.

With consideration of the GG-behavior, decision of γ_d can now be described as an optimization problem as follows (**P**lanning **P**roblem for γ , in short **PP**-γ):

To find γ^* in U (23) such that Min $J(\gamma)$
And satisfying $|\gamma| \le \|\boldsymbol{K}_1\|^{-1} a_{\max}$.

Let

$$J(\gamma) = k_a\gamma^2 + k_b(\gamma-\gamma_{dG})^2 + k_c sat(\|\boldsymbol{K}_1\|^{-1} a_{\max}) \tag{24}$$

where $sat(\gamma,\Gamma) = \begin{cases}0 & |\gamma|\le\Gamma\\ (\gamma-\Gamma)^2 & \text{else}\end{cases}$, and k_a,k_b,k_c>0, $k_a + k_b = 1$, k_c should be enough large, γ_{dG} is the desired steering angle γ_d for GG-behavior. Under the evaluation function of (24), the decision-making of PP-γ can be formulated to be

$$\gamma_d = \arg\min_{\gamma\in U}(J(\gamma)) \tag{25}$$

In (25), the CA-behavior, GG-behavior and the dynamic constraints of the mobile robot are all considered. Different k_a and k_b lead to a different tradeoff between the CA-behavior and GG-behavior. In order to try to guarantee the safety of the mobile robot and no local minima are encountered, we can let $k_a << k_b$. Note that the following equation is hold:

$$\arg\min_{\gamma}(J(\gamma))$$
$$= \arg\min_{\gamma}\{k_a\gamma^2 + k_b(\gamma-\gamma_{dG})^2 + k_c sat(\|\mathbf{K}_1\|^{-1} a_{\max})\}$$
$$= \arg\min_{\gamma}\{(\gamma - k_b\gamma_{dG})^2 + k_c sat(\|\mathbf{K}_1\|^{-1} a_{\max})\}$$

Hence, (24) can also be substituted by

$$J(\gamma) = (\gamma - k_b\gamma_{dG})^2 + k_c sat(\|\mathbf{K}_1\|^{-1} a_{\max}) \qquad (26)$$

5. Simulations

In this section, simulations are provided to illustrate our method. Any dynamic model of an omni-directional mobile robot can be used in the simulations. Parameters of the mobile robots used in simulations are: $a_{\max}$=0.5 m/s^2, $V_{\max}$=0.5 m/s, $\omega_{\max}$ =0.1 rad/s, the radius of the robot is r =0.3 m. The effective detecting radius of the sensors is 1.5 m. Velocity of the moving obstacles, if any, is 0.35m/s. T_1 is chosen to be the planning period, and let T_1=0.1s. Moreover, let k_a=0.9, k_b=0.1, k_c=5 (in (24) and (26)), k_s=100 (in (20)).

Specially, we assume that, only the distance between the mobile robot and a static obstacle is less than 1 m, then the CA-behavior with respect to this static obstacle is adopted. Additionally, in order to remove the oscillation on the trajectory and guarantee the safety when the mobile robot goes along the boundary of an obstacle, the following strategy is used:

(1) If $|\gamma| \le \delta$, let $\gamma = 0$, where δ is a small positive number.

Simulations are given under different situations in order to illustrate our method (Figure 3-4). In Figure 3, the environment is static with "SIA"-shape obstacles. The result shows that the mobile robots can coordinate to avoid collision with each other in this environment when they meet, and they can also navigate the U-shape obstacles without being trapped in local minima. Note that wall-following-like behavior is automatically generated in this case. Figure 4 shows a more complex environment, in which there are not only static obstacles but also two moving obstacles. The robots can effectively avoid collision with the unknown moving obstacles.

From the simulation, it can be seen that, (1) The trajectories planned for the mobile robot is smooth, and there are no local minima encountered Shapes of obstacles can be arbitrary, and no knowledge is required about the boundary or velocity of obstacles. (2) All the variables needed in the decision-making of **PP-**γ are in the local coordinates, and only local knowledge of the environment is needed. (3) Our method can be adaptable to dynamic environments, it has fast response to moving obstacles. (4) Our method can make different mobile robots coordinate to avoid collision with each other without "dead-lock".

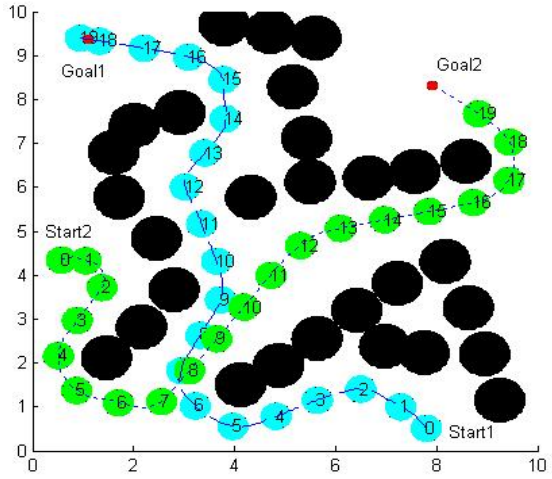

Figure 3. Two mobile robots are moving in an uncertain static environment with "SIA"-shape obstacles.

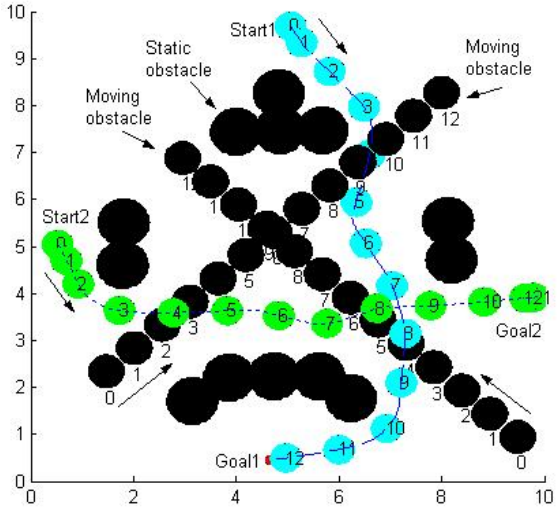

Figure 4. Two mobile robots are moving in a uncertain complex environment with static and moving obstacles.

6. Conclusions

The fundamental behaviors in motion planning of mobile robots are regarded as dynamic processes, and thus are modeled and controlled for motion planning purpose. Control of the behavior dynamics is shown to

be an acceleration control problem of the mobile robot. The behavior dynamics and the robot's dynamics are formulated into an integrated planning-and-control system. The motion-planning problem is thus transformed into a sensor-based control problem. The stability of a desired behavior can be guaranteed. By controlling and modeling of the behavior dynamics, a unique insight to the motion-planning problem is provided. Our method can effectively consider the robot dynamics into the motion-planning problem, use only relative coordinates and local knowledge of the environment, and respond quickly to obstacles of arbitrary shape. It should be noted that, behavior dynamics may also be used in some other motion control problems of robots such as formation control, cooperation of multi-robots, tele-operation, etc. Further study will focus on these problems.

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Alfalfa Drying Properties and Technologies – in Review

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Abstract: Fresh alfalfa must be dried to moisture contents of 8~10% for storage and processing. However, the physical and compositional characteristics of each component differ greatly. The drying properties of alfalfa were studied, which obtained some meaningful results. Dimensional analysis method was used to design rotary dryer and determine the drying parameters. [Nature and Science. 2004;2(4): 65-67].

Key Words: alfalfa; dry; property

1. Introduction

Alfalfa (*Medicago satica*, L) that is often called "Queen of forages" is the most important forage crop species in the world. In addition to being an excellent source of protein, vitamins and minerals, alfalfa is important for improving the soil. Good quality alfalfa hay contains digestible fibers and a useful range of minerals and vitamins. Since 1970 onwards, the processing of alfalfa to produce products like pellets and cubes is increasing due to ease of transportation and handling of these products.(Rotz C.A, 1984)

Fresh alfalfa must be dried to moisture contents of 8~10% for processing. The most popular form of artificial drying is in a chopped form in rotary dryers. The crop is mowed, conditioned by crushing or crimping of the stems, and the plant material is laid in the swath for drying. The partially dried forage is picked up and cut into small pieces. Fresh alfalfa may also be cut into pieces immediately after harvest. Alternatively, alfalfa is harvested and allowed to cure in the field (sun-cure) to balable moisture content of about 20 to 40%. These bales are then brought to the plant, chopped into 50 mm lengths and dried further to less than 10% for further processing. Bales are also produced with hay having 40% moisture and these bales are dried in a barn dryer to safe storage moisture of less than 15%. The dales may be fed to animal's long hay or processed in the form of cubes and dense bales for export market.(Hanson A, 1988)

2. Alfalfa drying properties

Unlike grain, alfalfa chop is not a homogenous material. It contains leaves, coarse stems, stems with leaves attached and fine stems. The physical and compositional characteristics of each component differ greatly. The leaves are thin and elliptical in shape, having large surface area for drying as well as the natural openings of the stomata, which aid moisture release. The leaves contain 30% protein compared to only 10% in the coarse stems. The coarse stems have hollow cross section with the external cuticle serving as a natural barrier. The fine stems, which comprise 10% of chop mass, are more succulent and approximate solid cylinders. (Patil RT, 1992)

Due to differences in their physical and chemical characteristics, each component must be studied separately for their dehydration characteristics to get a clear understanding of the drying operation of alfalfa and to better monitor the quality of the end product affected by the drying process. At present, the following research results were obtained.

It was demonstrated that at 60℃, alfalfa leaves were faster 2.5 times than stems to reach to the same moisture level of 8%. It may be therefore advisable to dry leaves and stems separately and later on try-mixing them in the proportion desired for further processing. Shorter the stem length is, and faster the drying rate of alfalfa is. The drying constant k was found to decrease logarithmically with the length of stem. It is therefore recommended that lowest possible stem length is used to achieve uniform drying whenever possible. The thin layer drying

characteristics of alfalfa indicates that the fine stems dry even at faster rate than the leaves. (Patil RT, 1992)

At lower air temperature of 40℃ drying takes place initially at a constant rate and at 60℃ in the falling rate. The falling rate at 40 and 60℃ can be divided further into two or three periods. However, above 100℃ the drying temperature rate initially were found to increase with moisture content. This phenomenon may be due to thermal damage to waxy surface, which helps in increasing the drying rate up to a critical level. After this, the rate of water replenishment to the surface was not as fast as evaporation and hence the drying takes place in the falling rate period. The drying rates were initially higher and reduced as the gradient of initial and final moisture lowered. The drying rates g/g-min at each 0.01 reduction in moisture was higher at higher temperatures. This indicates that unlike other food grains the casehardening effect was not experienced in alfalfa at high temperature drying. In fact, the thermo-mechanical damage to the cuticular layer helps in increasing the drying rate and it was therefore possible to use higher temperatures.(Patil RT, 1994)

The drying behavior of alfalfa showed the rate of moisture migration increased with increased drying air temperature, and like food grain the case hardening effect was not experienced. This could be attributed to its fibrous and exposed microstructure to dying environment and thermo-mechanical damage to the outer surface. Considerable changes in the physical attributes were found during drying which need to be considered during designing the dryer for alfalfa. Alfalfa can be dried up to 175℃ without appreciable damage to its physical structure.

At air temperatures above 300℃, the extremely rapid drying of chop in thin layer allowed the chop to burn after a certain exposure period which puts limits on the allowable exposure time of alfalfa at those temperature up to 1000℃ are used. Though the large throughput in rotary dryers produces the cooling effect due to evaporation of moisture, it is possible that some material may get exposed to excessively higher temperature for longer times.(Patil RT, 1994)

The typical drying curves for alfalfa chop and also goodness of fit at various drying air temperatures at the difficult initial moisture content. The non-linear relationship between moisture ratio with drying time was up to 200℃ for chop at initial moisture content of 3.10. Chops at initial moisture of also showed a nonlinear relationship up to 200℃. At moisture contents of 0.69 and 0.26, the nonlinear relationship was up to 200℃and 150℃ drying air temperatures, respectively. For the rest of the conditions, the relationship was linear.(Mujumdar AS, 2000)

The alfalfa protein is highly tolerant to heat. Research results show that protein solubility did not decrease up to 150℃ for leaves and 100℃ for stems. Damage to protein is significant at 150℃ for stems at 200℃ for leaves.(Patil RT, 1993)

Crushing chopped alfalfa at lower initial moisture contents (20% and 40%) expedited the drying rates. Crushing three times between longitudinally grooved rollers improved the drying rates However, increasing-crushing frequency beyond three times did not improve drying rates. The use of potassium carbonate as a chemical treatment on fresh alfalfa was effective in reducing the drying time by about 50% over the control, but chemical treatment did not the drying times of pre-wilted alfalfa due to the increased moisture content of the treated sample. Treatments with either steam or urea or a combined mechanical-chemical treatment did not reduce drying times because these treatments increase the initial moisture content of the forage samples.(Sokhansank S, 1996)

3. Alfalfa drying technologies

Rotary dryers are normally employed in the chemical and pharmaceutical industry but also are used to dry agricultural products like alfalfa. A rotary dryer basically consists of a cylindrical shell that rotates around its longitudinal axis. The inside of the shell is equipped with lifting flights. The material to be dried is introduced in a continuous way through one end of the dryer. The hot air is also introduced in the dryer where it contacts the products to be dried. Material is lifted by the flights during the rotating movement and dislodged falling back as it cascades through the hot air stream. The air provides the necessary heat to drive off the water in the product by vaporization. The product that passes through a rotary dryer has a more intimate contact with the air than in other types of dryers and so the drying is achieved in a more uniform way. As an essential dryer, the rotary dryer is widely used in the alfalfa drying process. Therefore, it is an important procedure to design a perfect rotary dryer for alfalfa drying, whose performance characters are the low energy

consumption and the high alfalfa quality. However, some rotary dryers were designed base on the other material such as the chemical material, to determine the structure and process parameters, which is unfit for the alfalfa drying.(wood HC, 1990)

Rotary drying is a complex process that involves not only thermal drying but also transport of the product. Rotary dryers constitute a highly non-linear system that is dependent both on time and position and whose modeling is very difficult. It is not easy to obtain the proper results to describe the alfalfa drying process in convention experiment design method such as quadratic rotary regress design method. Dimensional analysis method was used to determine the variables which reduce significantly the amount of experimental data that must be collected. It is based on the premise that physical quantities have dimensions and that changing the units measuring dimensions does not alter physical laws. Thus, the phenomenon under investigation can be described by a dimensionally correct equation among the variables. A dimensional analysis provides qualitative information about the model relevant to rotary drying process. It is especially important when it is necessary to conduct experiments in the modeling process because the method is helpful in testing the validity of including or neglecting a particular factor, in reducing the number of experiments to be conducted to make predictions, and in improving the usefulness of the results by providing alternatives for the parameters employed to present them. Dimensional analysis has proven useful in physics and engineering for many years and now even plays a role in study of the life sciences, economics, and operations research. Dimensional analysis method was used in the modeling process in a rotary alfalfa dryer to increase the efficiency of an experimental design.

The Jiang-theorem and G. Murphy-Jiang theorem in dimensional analysis are characteristic of less experiment number of times and easy operation and reasonable results.

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Regression Analysis for Mapping Dynamic Trait QTL in Outbred Population

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Abstract: By using Legendre polynomials to model dynamic changes of each genetic effect, a mathematic model was constructed for mapping dynamic trait loci. Other than the number of estimated effects increasing several times, there were no differences between the mapping principles for dynamic trait and the general quantitative trait loci underlying the same genetic design. Therefore, given the prerequisite that, residual errors at each observed point have the same distribution and are independent from each other, it is feasible to use regression analysis (REG) as an alternative of maximum likelihood (ML) for simplifying the estimated model parameters and detects QTL for dynamic traits. The new strategy for mapping dynamic trait loci is developed in the context of an outbred population with three-order Legendre polynomial as a sub-model. The properties of regression analysis are demonstrated and compared with maximum likelihood via replicated Monte Carlo simulations. The factors considered include individual number, test-day frequency, marker frequency and accumulative heretability, the level combinations of which were based on orthogonal table. It showed that REG performs equally well with ML, but deficient in accuracy and precision of residual variance estimating. The detection power of both methods depends greatly on size of accumulative heretability. [Nature and Science. 2004;2(4):68-78].

Key Words: dynamic trait; QTL mapping; Legendre polynomial; regression analysis; simulation

1. Introduction

The growth development of tissues and organs and change of performance with time of life or certain quantitative factors belong to the category of dynamic trait, which is of major interest to developmental genetics in plant and animal breeding studies.

Existing methods for genetic mapping of a dynamic trait can be synoptically divided into two classes: dynamic point-based method and dynamic model-based method. The dynamic point-based method treated each dynamic point as a different trait and genetically map dynamic trait loci by using separate analysis (Cheverud *et al.* 1996; Nuzhdin *et al.* 1997; Verhaegen *et al.* 1997; Emebiri *et al.* 1998; Wu *et al.* 1999), mutitrait analysis (Jiang & Zeng 1995; Korol *et al.* 1995; Ronin *et al.* 1995; Eaves *et al.* 1996; Knott and Haley 2000) or conditional analysis for these phenotypes at each dynamic points (Yan et al, 1998a, b; Wu et al, 2002). However, this analysis approach can just provide partial estimates of genetic control over dynamic traits, because it was not possible to capture the whole information about infinite dynamic points in the dynamic process. Also, it is required to observe in equilibrium phenotypes at dynamic points from each individual except separate analysis. A dynamic model-based method proposed recently attempted to detect QTL affecting the changing process of dynamic trait via analyzing the phenotypic function (curve) on time in life and other quantitative factors. In papers just published, Rodriguez-Zas *et al*. (2002) developed a longitudinal-linkage analysis approach for mapping QTL affecting the shape and scale of lactation curves for production and health traits in dairy cattle in outbred population. By combining growth models with QTL mapping, W. R. Wu et al (2002) proposed an approach of QTL mapping based on growth models. Both two approaches are archived by two steps: the first is to fit the dynamic curve of each individual or line with a theoretical or empirical mathematical model and then map QTLs based on the estimated model parameters. As a rule, two-step estimation

performs lower statistical power because the estimated errors of model parameters are not considered in the second step. Based on functional mapping strategy (Ma *et al* 2002; Wu et al 2002; 2003), the models for dynamic trait mapping QTL were established by using Logistic curves to describe the effects of QTL genotypes on the growth process of a forest tree. Although effects of QTL genotypes can be estimated by one step, it is difficult to estimate the additive and interaction effects of QTL and fail to extent to arbitrary population for estimating simultaneously multiple QTL genetic effects. Enlightened by the random regression model (Henderson 1982; Schaeffer & Dekkers, 1994; Schaeffer, 2002), said the Legendre polinomial may be nested into each QTL effect in general QTL mapping model to describe the change of QTL effects on dynamic traits and so the new mathematic model for mapping dynamic trait loci can be constructed for mapping one or multiple QTLs in various population (Yang et al, 2003).

Maximum likelihood, implemented with the EM algorithm, was used at the beginning of interval mapping advent. It can fully capture the information of data distribution, but could not be implemented in complex data structure because of the difficulty in computations. Haley and Knott (1992) introduced regression analysis method to interval mapping by substituting the probabilities of each QTL genotype under the markers for the indicator variable of each genotype and proved that it can save much time in computation and get similar results as ML. Other than the number of estimated effects increased several times, there were no differences between the mapping principles for dynamic trait and the general quantitative trait under the same genetic design. The objective in this study is to apply regression analysis to simplify and estimate model parameters and detect QTL for dynamic traits and also compare with maximum likelihood, simulation experiments in outbred populations.

2. Mapping principle

2.1 Genetic design

The population exampled to demonstrate the application of QTL mapping for dynamic trait loci in animals in this study is the full-sibs of outbred cross, which is most general in animal populations. Under Mendelian inheritance laws, there are four possible genotypes denoted by $Q_1^sQ_1^d$, $Q_1^sQ_2^d$, $Q_2^sQ_1^d$ and $Q_2^sQ_2^d$. If Legendre polynomial is used to model the genetic change with time or other factors of dynamic trait or the effect of QTL, the statistical model describe the phenotypic value of individual i at j test-day may be

$$y_{ij} = \mathrm{T}'_{ij}\mu + X_{i1}\mathrm{T}'_{ij}\mathrm{g}_1 + X_{i2}\mathrm{T}'_{ij}\mathrm{g}_2 + X_{i3}\mathrm{T}'_{ij}\mathrm{g}_3 + X_{i4}\mathrm{T}'_{ij}\mathrm{g}_4 + e_{ij}$$
$$= \mathrm{T}'_{ij}\mu + \sum_{k=1}^{4} X_{ik}\mathrm{T}'_{ij}\mathrm{g}_k + e_{ij}$$

$i = 1, 2, \cdots, n$; $j = 1, 2, \cdots, m$; $k = 1, 2, 3, 4$.

Where, $\boldsymbol{\mu}$ is the regression coefficient vector of the Legendre polynomial for population means; $\mathbf{T}'_{ij} = \begin{bmatrix} 1 & P_1(\tau_{ij}) & \cdots & P_s(\tau_{ij}) \end{bmatrix}'$ with τ and s representing standardized time t and the order of Legendre polynomial selected, respectively; X_{ik} is the indicator variable of QTL genotype, valued 1 when the genotype of individual i is the k or 0 when the other. g_k denotes vector of fixed regression effects corresponding to genotype k.

2.2 Statistical method

Maximum likelihood. The phenotypes of the traits at all time points for each genotype group follow a multivariate normal distribution:

$$f_k(y_{ij}) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left[-\frac{(y_{ij} - \mathrm{T}'_{ij}\mu - \mathrm{T}'_{ij}\mathrm{g}_k)^2}{2\sigma^2} \right]$$

Actually, the genotype of individual i is unobservable, and it must be inferred by markers as conditional probability. Then the likelihood function of the outbred cross with m-dimensional measurements can be represented by a multivariate mixture distribution:

$$L(\mu, \mathrm{g}, \sigma^2) = \prod_{i=1}^{n}\prod_{j=1}^{m}\left[\sum_{k=1}^{4} p_{ik} f_k(\mathrm{y}_{ij}) \right]$$

The unknown parameters about QTL can be estimated using maximum likelihood implementing the EM algorithm. The steps of EM algorithm can be

recapitulated as

(1) Set the initial values of each parameter $\Omega^{(0)} = [\mu^{(0)}\ g^{(0)}\ \sigma^{2(0)}]$;

(2) Calculate the posterior probabilities of each QTL genotype $p_{ik} = \frac{p_{ik} f_k(\mathrm{y}_{ij})}{\sum_{k=1}^{4} p_{ik} f_k(\mathrm{y}_{ij})}$;

(3) E-step: calculate the expectations under the posteriors;

(4) M-step: estimate the MLE of each parameter.

Let $\mathrm{g}' = [\mathrm{g}_1 \quad \mathrm{g}_2 \quad \mathrm{g}_3 \quad \mathrm{g}_4]$,

$$\mathrm{A} = \begin{bmatrix} \sum_{i=1}^{n}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p'_{i1}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p'_{i2}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p'_{i3}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p'_{i4}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} \\ & \sum_{i=1}^{n}p'_{i1}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij_{ij}} & 0 & 0 & 0 \\ & & \sum_{i=1}^{n}p'_{i2}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & 0 & 0 \\ & & & \sum_{i=1}^{n}p'_{i3}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & 0 \\ \textit{sym.} & & & & \sum_{i=1}^{n}p'_{i4}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} \end{bmatrix}$$

$$\mathrm{C}' = \begin{bmatrix} \sum_{i=1}^{n}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} & \sum_{i=1}^{n}p'_{i1}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} & \sum_{i=1}^{n}p'_{i2}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} & \sum_{i=1}^{n}p'_{i3}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} & \sum_{i=1}^{n}p'_{i4}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} \end{bmatrix}$$

then $[\mu^{(1)} \vdots\ \mathrm{g}^{(1)}] = \mathrm{A}^{-1}\cdot\mathrm{C}$ $\quad \hat{\sigma}^{2(1)} = \frac{1}{n}\sum_{i=1}^{n}\sum_{k=1}^{4}p'_{ik}\sum_{j=1}^{m}(y_{ij} - \mathrm{T}_{ij}\mu^{(1)} - \sum \mathrm{T}_{ij}\mathrm{g}_k^{(1)})^2$.

With $\mathbf{\Omega}^{(0)}$ substituted by $\mathbf{\Omega}^{(1)}$, iterations are then made between step (2), (3) and (4) and terminated when a predetermined criterion is satisfied. The final estimates are to be the MLEs.

In practical computations, the QTL position parameter θ can be viewed as fixed because a putative QTL can be searched at every 1 or 2 cM on a map interval bracketed by two markers throughout the entire linkage map. The amount of support for a QTL at a particular map position is often displayed graphically through likelihood profiles. The position of the largest and most significant is considered the most possible that the putative QTL lying.

Regression analysis. Different from ML, the indicator variables for each genotype in the statistical model are first replaced by their conditional expectations given marker information, i.e.,

$$y_{ij} = \mathrm{T}'_{ij}\mu + \sum_{k=1}^{4}\hat{X}_{ik}\mathrm{T}'_{ij}\mathrm{g}_k + e_{ij}\ .$$

Where, $\hat{X}_{ik} = E(X_{iki} \mid \mathrm{MN})$, MN represented the flanking markers. The estimation and statistical test of QTL effects can be accomplished using conventional multiple regression analysis by treating $\hat{X}_{ik}$ as observed values.

The conditional probabilities of each QTL genotype, $\Pr(\mathrm{Q} \mid \mathrm{MN})$, were calculated by multipoints estimation method (Rao and Xu, 1998; Xu et. 1998).

Let $\Pr(Q \mid \mathrm{MN}) = p_{ik}$, then $\hat{X}_{ik} = E(X_{ik} \mid \mathrm{MN}) = [p_{i1} \quad p_{i2} \quad p_{i3} \quad p_{i4}]$, and

$$\mathrm{A}=\begin{bmatrix} \sum_{i=1}^{n}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i1}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i2}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i3}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i4}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} \\ & \sum_{i=1}^{n}p_{i1}^{2}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i1}p_{i2}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i1}p_{i3}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i1}p_{i4}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} \\ & & \sum_{i=1}^{n}p_{i2}^{2}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i2}p_{i3}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i1}p_{i4}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} \\ & & & \sum_{i=1}^{n}p_{i3}^{2}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} & \sum_{i=1}^{n}p_{i3}p_{i4}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} \\ sym. & & & & \sum_{i=1}^{n}p_{i4}^{2}\sum_{j=1}^{m}\mathrm{T}'_{ij}\mathrm{T}_{ij} \end{bmatrix}$$

$$\mathrm{C}'=\begin{bmatrix} \sum_{i=1}^{n}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} & \sum_{i=1}^{n}p_{i1}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} & \sum_{i=1}^{n}p_{i2}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} & \sum_{i=1}^{n}p_{i3}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} & \sum_{i=1}^{n}p_{i4}\sum_{j=1}^{m}\mathrm{T}'_{ij}y_{ij} \end{bmatrix}$$

The estimates of each parameter can be calculated by

$$[\mu\vdots\ \ \mathrm{g}]=\mathrm{A}^{-1}\cdot\mathrm{C}$$

$$\hat{\sigma}^{2}=\frac{1}{nm-nk}\sum_{i=1}^{n}\sum_{j=1}^{m}(y_{ij}-\mathrm{T}_{ij}\mu-\sum_{k=1}^{4}p_{ik}\mathrm{T}_{ij}\mathrm{g}_{k})^{2}$$

Ulteriorly, the means to detect the possible position of the putative QTL in linkage map is the same as the ML's.

2.3 Estimation for genetic effects

In an outbred family, the genotypic value of a progeny can be partitioned according to the following genetic model,

$$g_{kij}=a_{ki}^{s}+a_{kj}^{d}+d_{kij}\quad(i=1,2;\ j=1,2).$$

Where a_{k1}^{s} and a_{k2}^{s} are the effects of the two alleles in the sire, a_{k1}^{d} and a_{k2}^{d} are the effects of the two alleles in the dam, and d_{kij} are the dominance deviations. Because these effects are not estimable, some constraints are required. These constraints are

$$\sum_{i=1}^{2}a_{ki}=\sum_{j=1}^{2}a_{kj}=\sum_{i=1}^{2}d_{kij}=\sum_{j=1}^{2}d_{kij}=0.$$

Under these constraints there are three independent estimable effects, which are conveniently defined as:

（1） $a_{k}^{s}=a_{k1}^{s}-a_{k2}^{s}$, the average effect of allelic substitution for the sire;

（2） $a_{k}^{d}=a_{k1}^{d}-a_{k2}^{d}$, the average effect of allelic substitution for the dam;

（3） $d_{k}=d_{k11}-d_{k21}-d_{k12}+d_{k22}$, the interaction between alleles of the sire and the dam (dominance effect).

The three genetic effects, $\gamma_{k}=[\mathrm{a}_{k}^{s}\quad\mathrm{a}_{k}^{d}\quad\mathrm{d}_{k}]$, are estimated by $\gamma_{k}=\mathrm{H}^{T}\mathrm{g}_{k}$.

Where

$$\mathrm{H}=\begin{bmatrix} \frac{1}{2} & \frac{1}{2} & 1 \\ \frac{1}{2} & -\frac{1}{2} & -1 \\ -\frac{1}{2} & \frac{1}{2} & -1 \\ -\frac{1}{2} & -\frac{1}{2} & 1 \end{bmatrix}.$$

2.4 Hypothesis tests

The hypothesis about the existence of a QTL affecting an overall process of dynamics can be formulated as

H_0: $\gamma_{k}=0$ H_A: $\gamma_{k}\neq 0$ (k=1,2,3)

That H_0 is rejected means there has a QTL existent in the map.

The test statistics for testing the hypothesis is calculated as the log-likelihood ratio (LR) of the full over reduced model:

$$\mathrm{LR}=-2\log\left[\frac{L(\tilde{\Omega})}{L(\hat{\Omega})}\right].$$

Where $\tilde{\Omega}$ and $\hat{\Omega}$ denote the ML estimates of the unknown parameters under H_0 and H_1, respectively.

3. Simulation studies

The properties of the method, especially unbiaseness, standard errors of the parameter estimation and statistical power, were investigated numerically via Monte Carlo simulations with a full-sib population. On a single chromosome segment of length 100 cM consider and simulate 6, 11 and 21 evenly spaced codominant markers covered,

respectively, and a single QTL was supposed locating at position 27 cM. The test-day of the data observed a range from 1 to 150. Alleles inherited at each position in individuals were generated by the linkage phase inherited from estimation.

Assume the effects of the QTL of dynamic trait were fixed in statistical model and described its different contribution to phenotypic values at different time points by three-orders Legendre polynomial. The first four terms of Legendre polynomial were

$$P_0(\tau)=1, \qquad P_1(\tau)=\tau,$$

$$P_2(\tau)=\frac{1}{2}(3\tau^2-1), \qquad P_3(\tau)=\frac{1}{2}(5\tau^3-3\tau)$$

Let the mean values of regression effect in population be 0, then the phenotypic value of individual *i* at time *j* can be calculated as follow according to QTL genotypes of each individual and test project of this population, given the additive effect (a_0^s for sire and a_0^d for dam) and dominance effects (d_0) of the parents and the residual variance σ_0^2.

$$y_{ij}=\frac{1}{2}(\mathrm{T}'_{ij}\mathrm{a}_0^s+\mathrm{T}'_{ij}\mathrm{a}_0^d)+\frac{1}{4}\mathrm{T}'_{ij}\mathrm{d}_0+\xi\cdot\sigma_0$$

Where, ξ is a random number of standardized normal distribution.

Given effect, a_0 ($\mathrm{a}_0^s=\mathrm{a}_0^d=a_0$) and d_0, the variance and covariance of each regression effect were calculated as $G_{a^s}=G_{a^d}=\frac{1}{4}\mathrm{a}'_0\mathrm{a}_0$, $G_d=\frac{1}{16}\mathrm{d}'_0\mathrm{d}_0$.

Combined with residual variance σ^2, the heredity in each point of the dynamic process can be calculated as

$$h_i^2=\frac{g(i)}{g(i)+\sigma_0^2}$$

Where, $g(t)=T_i(G_a+G_d)T_i'$ with

$$\mathrm{T}'_i=\begin{bmatrix}1 & P_1(t_i) & \cdots & P_s(t_i)\end{bmatrix}'.$$

The phenotypic value, additive effect value, dominance effect value at each dynamic points were calculated under each given genetic regresson effect, $\mathrm{a}'_0=\begin{bmatrix}1.58 & 0.65 & -1.02 & 1.65\end{bmatrix}$ and $\mathrm{d}'_0=\begin{bmatrix}1.77 & 0.36 & -0.71 & 1.27\end{bmatrix}$, and graphically represented in Figure 1a and 1b.

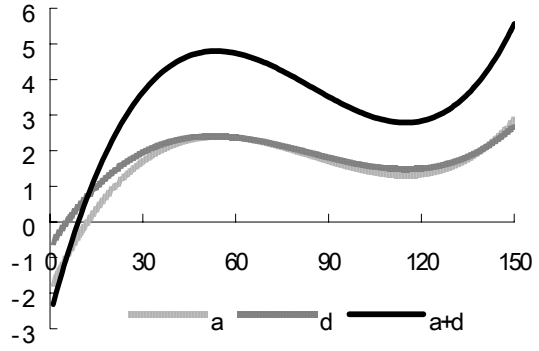

Figure 1a. Dynamic curve of total genetic value, additive and dominance effect value

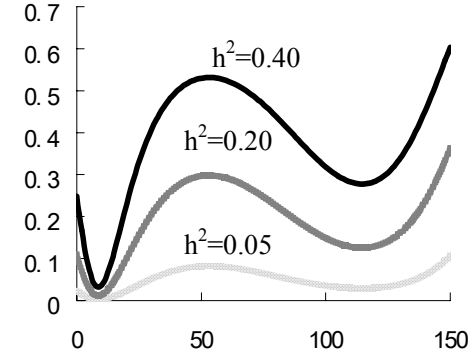

Figure 1b. Heritability change at each dynamic point under three accumulative accumulative heretability levels

The factors considered included individual number, test-day frequency, marker number and heredity level and each taken three levels as 0.05, 0.20 and 0.40 were taken in accumulative heretability; 100, 300 and 500 in individual; 6, 11 and 21 in marker; 5, 10 and 15 in test-day frequency.

Since the combinations of factors considered were too much, the experiments of simulation were arranged according to orthogonal table (Table 1). The simulation was repeated 100 times for each situation. The mean estimate and the standard error of an estimated parameter, calculated from 100 replicated simulations, were showed in Table 2-4.

The statistical power was determined by counting the proportion of the number of runs (over 100 replicates) with test statistic values greater than an empirical threshold. The empirical threshold value was obtained by choosing the 95-th and 99-th percentiles of the highest test statistic over 1000 additional runs under the null model (no QTL is segregating). Table 2 shows that ML method provides slightly higher threshold values than regression analysis, but these critical values hardly depend on each factor.

Table 1. Experimental factor and its combinations

Groups	Factors			
	Heretabilities	Marker frequencies	Individual numbers	Test-day frequencies
1	0.05	6	100	5
2	0.05	11	300	10
3	0.05	21	500	15
4	0.2	6	300	15
5	0.2	11	500	5
6	0.2	21	100	10
7	0.4	6	500	10
8	0.4	11	100	15
9	0.4	21	300	5

Table 2. Empirical critical values of the test statistics in different combinations（95%, 99%）

Groups	Methods	95%	99%
1	ML	21.72	24.24
	REG	21.03	23.50
2	ML	19.68	21.53
	REG	19.54	21.38
3	ML	18.87	20.64
	REG	18.86	20.64
4	ML	18.34	19.98
	REG	18.34	19.98
5	ML	19.53	20.00
	REG	18.41	19.88
6	ML	19.98	21.96
	REG	19.85	21.83
7	ML	17.95	19.71
	REG	17.86	19.58
8	ML	19.57	21.52
	REG	19.59	21.57
9	ML	19.44	21.33
	REG	19.44	21.33

Table 3 showed the estimates (and standard deviation) of QTL position, residual variance and the powers of regression analysis for detection compared with ML in different combinations. Generally speaking, the two methods show virtually no differences, which is consistent with the observation of Xu (1998) and Haley and Knott's (1992) comparison of ML and REG in mapping QTL for general quantitative trait. The methods behave as expected: a high level factor tends to produce an unbiased estimate of the QTL position and small estimation errors. Both methods perform well in detection power, which may reach 98% when individual number over 300 even only the 5% variation of the trait was controlled by the QTL. The power of QTL detection in regression analysis was above 65% when the individual number was just 100. If only analysis single records of a trait, the number of individual needed that ensure detection power be of 95% would be 500 in the same situation. It sufficiently shows the complement of test-day frequency and individual number.

Both methods perform well in estimating the residual variance with little estimation error and high precision under every combination. Although ML behaves better than REG in the same situation, the estimates of the two methods are both tending to the true values as the sample size, which defined as the product of individual number and test-day frequency increasing.

Table 3. Estimates of QTL Position, Residual Variance and the Powers for Detection Calculated from 1000 Repeated Simulations

Groups	Methods	cM	σ^2	Statistical power	
				α =0.05	α =0.01
1	ML	28.735(0.541)	33.106(0.0806)	79	69
	REG	29.188(0.537)	36.949(0.0775)	77	71
2	ML	27.249(0.0784)	34.895(0.0294)	100	100
	REG	27.277(0.0801)	35.047(0.0308)	99	98
3	ML	26.988(0.0314)	35.018(0.0181)	100	100
	REG	26.988(0.0311)	35.017(0.0191)	100	100
	True	27.00	7.379		
4	ML	27.179(0.0633)	7.350(0.00597)	99	99
	REG	27.168(0.0787)	7.283(0.00719)	99	99
5	ML	27.017(0.0383)	7.329(0.00676)	100	100
	REG	27.071(0.0412)	7.336(0.00857)	100	100
6	ML	26.913(0.0576)	7.262(0.0104)	100	100
	REG	26.889(0.0602)	7.298(0.0144)	100	100
	True	27.00	2.767		
7	ML	26.880(0.0415)	2.758(0.00224)	100	100
	REG	26.786(0.0625)	2.717(0.00394)	100	100
8	ML	27.018(0.0601)	2.728(0.00376)	100	100
	REG	27.035(0.0654)	2.635(0.00818)	100	100
9	ML	27.001(0.0242)	2.760(0.00199)	100	100
	REG	27.007(0.0275)	2.7037(0.00628)	100	100

Table 4. Mean Estimates and Standard Deviations (in parentheses) of Effects of QTLCalculated from 1000 Repeated Simulations

Groups	Methods	a_0^s	a_1^s	a_2^s	a_3^s	a_0^d	a_1^d	a_2^d	a_3^d	d_0	d_1	d_2	d_3
	True	1.58	0.65	-1.02	1.65	1.58	0.65	-1.02	1.65	1.77	0.36	-0.71	1.27
1	ML	1.55	0.64	-0.98	1.65	1.61	065	-1.02	1.62	1.68	0.35	-0.65	1.13
		(0.024)	(0.036)	(0.045)	(0.057)	(0.025)	(0.037)	(0.045)	(0.057)	(0.058)	(0.092)	(0.102)	(0.138)
	REG	1.54	0.64	-0.98	1.68	1.58	0.66	-1.00	1.64	1.70	0.39	-0.63	1.07
		(0.023)	(0.037)	(0.045)	(0.057)	(0.023)	(0.037)	(0.043)	(0.057)	(0.057)	(0.086)	(0.104)	(0.137)
2	ML	1.58	0.63	-1.01	1.66	1.58	0.64	-1.01	1.65	1.78	0.32	-0.74	1.34
		(0.008)	(0.013)	(0.015)	(0.018)	(0.008)	(0.013)	(0.016)	(0.018)	(0.016)	(0.027)	(0.033)	(0.040)
	REG	1.58	0.63	-1.01	1.66	1.58	0.64	-1.01	1.65	1.78	0.33	-0.75	1.34
		(0.008)	(0.013)	(0.015)	(0.018)	(0.008)	(0.013)	(0.016)	(0.018)	(0.016)	(0.027)	(0.033)	(0.040)
3	ML	1.59	0.66	-1.02	1.66	1.58	0.65	-1.05	1.66	1.76	0.37	-0.71	1.30
		(0.005)	(0.008)	(0.010)	(0.012)	(0.005)	(0.008)	(0.010)	(0.012)	(0.009)	(0.016)	(0.021)	(0.023)
	REG	1.59	0.65	-0.99	1.65	1.59	0.67	-1.06	1.63	1.77	0.38	-0.65	1.30
		(0.004)	(0.008)	(0.011)	(0.011)	(0.005)	(0.084)	(0.095)	(0.011)	(0.010)	(0.015)	(0.021)	(0.023)
4	ML	1.58	0.64	-1.02	1.63	1.58	0.65	-1.02	1.65	1.76	0.35	-0.70	1.27
		(0.004)	(0.005)	(0.007)	(0.007)	(0.004)	(0.005)	(0.006)	(0.008)	(0.007)	(0.011)	(0.015)	(0.017)
	REG	1.58	0.65	-1.02	1.63	1.59	0.65	-1.02	1.65	1.76	0.35	-0.70	1.28
		(0.004)	(0.005)	(0.007)	(0.008)	(0.005)	(0.006)	(0.007)	(0.008)	(0.010)	(0.012)	(0.016)	(0.019)
5	ML	1.58	0.65	-1.01	1.66	1.58	0.65	-1.03	1.66	1.78	0.38	-0.71	1.30
		(0.004)	(0.006)	(0.008)	(0.010)	(0.004)	(0.006)	(0.008)	(0.009)	(0.008)	(0.013)	(0.016)	(0.020)
	REG	1.58	0.65	-1.02	1.66	1.58	0.65	-1.03	1.66	1.78	0.38	-0.71	1.29
		(0.004)	(0.006)	(0.008)	(0.010)	(0.004)	(0.006)	(0.008)	(0.009)	(0.008)	(0.014)	(0.016)	(0.020)
6	ML	1.57	0.65	-1.03	1.66	1.58	0.64	-1.02	1.64	1.78	0.35	-0.73	1.29
		(0.006)	(0.010)	(0.012)	(0.141)	(0.006)	(0.010)	(0.012)	(0.014)	(0.012)	(0.020)	(0.025)	(0.029)
	REG	1.57	0.65	-1.03	1.66	1.58	0.64	-1.02	1.64	1.78	0.35	-0.73	1.29
		(0.006)	(0.010)	(0.012)	(0.014)	(0.006)	(0.010)	(0.012)	(0.014)	(0.013)	(0.020)	(0.025)	(0.029)
7	ML	1.58	0.65	-1.02	1.64	1.58	0.65	-1.03	1.65	1.77	0.36	-0.71	1.28
		(0.002)	(0.003)	(0.004)	(0.004)	(0.002)	(0.003)	(0.004)	(0.004)	(0.004)	(0.007)	(0.008)	(0.009)
	REG	1.58	0.65	-1.02	1.65	1.58	0.66	-1.02	1.66	1.79	0.36	-0.71	1.28
		(0.003)	(0.003)	(0.004)	(0.006)	(0.004)	(0.004)	(0.005)	(0.006)	(0.010)	(0.008)	(0.010)	(0.014)
8	ML	1.57	0.65	-1.01	1.63	1.58	0.66	-1.02	1.63	1.77	0.36	-0.72	1.27
		(0.003)	(0.005)	(0.007)	(0.008)	(0.003)	(0.005)	(0.007)	(0.008)	(0.006)	(0.011)	(0.014)	(0.015)
	REG	1.58	0.64	-1.01	1.63	1.58	0.66	-1.03	1.64	1.78	0.36	-0.73	1.29
		(0.004)	(0.005)	(0.007)	(0.008)	(0.004)	(0.005)	(0.007)	(0.008)	(0.007)	(0.011)	(0.014)	(0.016)
9	ML	1.58	0.65	-1.02	1.65	1.58	0.65	-1.03	1.65	1.77	0.37	-0.71	1.29
		(0.002)	(0.003)	(0.004)	(0.004)	(0.002)	(0.003)	(0.004)	(0.004)	(0.003)	(0.006)	(0.007)	(0.008)
	REG	1.57	0.64	-1.02	1.66	1.58	0.65	-1.02	1.64	1.77	0.37	-0.70	1.25
		(0.003)	(0.005)	(0.006)	(0.007)	(0.003)	(0.005)	(0.006)	(0.008)	(0.006)	(0.010)	(0.012)	(0.015)

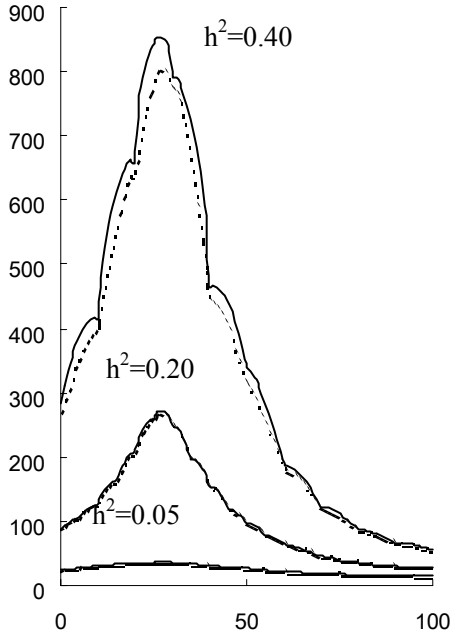

Figure 2a. Comparison of the likelihood ratio test statistic profiles of REG (dotted line) with ML (solid line) under three accumulative levels heritability with 100 individuals

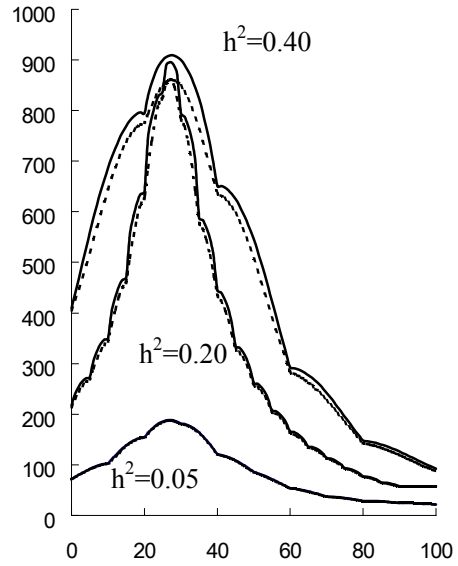

Figure 2b. Comparison of the likelihood ratio test statistic profiles of REG (dotted line) with ML (solid line) at three levels of QTL heritability with with 300 individuals

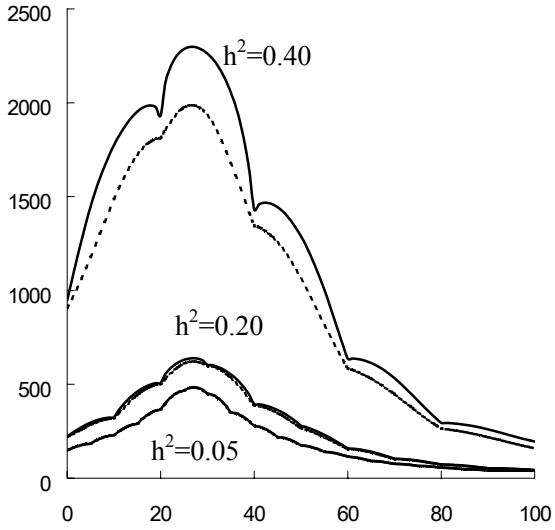

Figure 2c. Comparison of the likelihood ratio test statistic profiles of REG (dotted line) with ML (solid line) at three levels of QTL heritability with 500 individuals

To compare the two methods in QTL detection, the likelihood ratio test statistic profiles were plotted against the chromosome position (Figure 2a-c). It is easily seen that the profiles of the two methods almost overlapped under the heritbility of low level. The test statistic of ML method seems apparently greater than that of REG method. Both methods could hardly detect the existence of QTL under low heritability, but both showed high ability in detection for the trait with high heritability.

4. Discussion and Conclusion

QTL mapping is a typical problem of the regression with uncertain independent variables. It is well known that ML is the optimal method for this problem because the distributions of the unknown independent variables are fully taken into account (Lander and Botstein 1989; Jansen 1994; Zeng 1994). Under normal residual distribution, the REG method can provide a first-order approximation to the ML in the sense that the unobserved independent variables replaced by their conditional expectations (Haley and Knott 1992; Xu 1998). Other than time number of estimated effects increased, there were no differences between the mapping principles for dynamic trait and the general quantitative trait loci by using Legendre polynomial to describe QTL genetic effects

underlying same genetic design. If residual variance of each observation point is supposed to be equal and of normal distribution, the unknown indicator variable for QTL genotypes can be replaced by its expectation and REG method could be used to estimate the parameter of QTL for mapping dynamic traits. The estimation for the residual variance tends to be slightly expanded whereas the unknown QTL genotypes simply replaced by their conditional probabilities given maker information.

The process of simulation analysis indicated that REG method is of absolute advantage in computational algorithm and speed and of certain detection power compared with the ML.

The results of simulation showed that REG performs equally well with ML, but deficient in accuracy and precision of residual variance estimating. The detection power of both methods depends greatly on heredity level, but the power of detection would be sufficient with individuals above 300, makers over 6 and test-day frequency at 5% or more even if in lower heritability.

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The Effect of Nitrogen Level on mMain Nutrient of Sugar Beet

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Abstract: The nitrogen content of sugar beet plant was significantly positive correlated with nitrogen amount used. At the beginning of beet growing, the distribution of nitrogen was mainly in leaves, but later the distribution rate of nitrogen increased in roots. However, the distribution rate of nitrogen in leaves was still on high level when treated with high amount of nitrogen, which would be against the accumulation of sugar. The contents of phosphorous and potassium of beet plant were also significantly positive correlated with nitrogen amount used, and nitrogen has obvious interaction effect with phosphorus and potassium. [Nature and Science. 2004;2(4):79-83].

Key Words: sugar beet; nitrogen metabolism; interaction effect

1. Introduction

Fertilizers are the main food for plant. Nitrogen is one of the three components in fertilizers, and also the sensitive element that affect the beet production and sugar content (Zhou, 1993). The study of nitrogen amount on beet production and sugar content will be important and significant (Qu, 1992). This article demonstrates the effect of nitrogen amount on main nutrients of sugar beet.

2. Materials and Methods

2.1 Species used in this experiment

Tianyan No.7, provided by Hulan sugar beet research institute, Heilongjiang Province, China.

2.2 Design of the experiment

Five treatments were used upon $P_{90}K_{90}$ (kg/hm^2): N_0, N_{60}, N_{120}, N_{180}, N_{240} (kg/hm^2). Random group design was used, which including five rows and four repeats. The length of row was 11 m, the width of row was 0.6 m and the plot area was 33 m^2. The basic fertility of soil was: organic matter 25.57 g/kg, total nitrogen 1.71 g/kg, total phosphate 0.78 g/kg, total potassium 24.2 g/kg, alkalized nitrogen 145.1 g/kg, instant phosphate 35.0 mg/kg, instant potassium 202 mg/kg and pH 6.9 (Xu, 1983; Zheng, 1984).

2.3 Measurement methods

Total nitrogen: distillation method.
Total phosphate: colorimetry.
Total potassium: flame photometer.

3. Results and analysis

3.1 The nitrogen contents of sugar beet plant and their distribution

The nitrogen contents of sugar beet plant and their distribution with different nitrogen level were shown in Table 1.

Table 1. The nitrogen contents of sugar beet plant and their distribution

Growing stages (Month.date)	Treatment	Nitrogen contents in beet plants		Nitrogen distribution in beet plant (%)	
		g/plant	%	leaves	roots
Seedling stage 5.30	N_0	0.90×10^{-2}	2.61	93.93	6.07
	N_{60}	1.00×10^{-2}	2.78	93.96	6.04
	N_{120}	1.10×10^{-2}	2.87	94.36	5.64

Continue Table 1:

	N_{180}	1.20×10^{-2}	2.96	94.40	5.60
	N_{240}	1.20×10^{-2}	2.98	94.62	5.38
Leaf fascicle formation stage 7.4	N_0	0.80	2.19	85.75	14.25
	N_{60}	1.01	2.28	86.04	13.96
	N_{120}	1.10	2.44	86.76	13.24
	N_{180}	1.26	2.65	87.51	12.49
	N_{240}	0.57	2.74	87.79	12.21
Epacme of root tuber 8.15	N_0	1.53	1.13	79.84	20.16
	N_{60}	2.05	1.33	81.19	18.81
	N_{120}	2.64	1.42	82.16	17.84
	N_{180}	3.04	1.50	82.56	17.44
	N_{240}	3.57	1.67	83.79	16.21
Sugar accumulation stage 9.3	N_0	1.24	0.70	69.12	30.79
	N_{60}	1.61	0.87	74.14	25.86
	N_{120}	2.10	1.06	78.03	21.97
	N_{180}	2.38	1.11	78.32	21.68
	N_{240}	2.84	1.32	81.19	18.81

There were different nitrogen contents absorbed in each organ of sugar beet plant. In all of the growth stage, the absolute nitrogen contents in leaves were higher than that in leafstalks and roots, while that in roots were higher than that in leafstalks. The nitrogen contents in leaves, leafstalks and roots were shown as unimodal curves. The maximum value of absolute nitrogen contents in leaves and leafstalks appeared at the middle of August, and that in roots were appeared at the beginning of September. The relative nitrogen contents of each organ with different treatments were shown the same regularity in the whole growth stage, that is, it was decreased from the beginning to the sugar accumulation stage. The relative nitrogen contents of each organ was leaves > roots > leafstalks.

With the increasing of nitrogen used, the nitrogen contents of each organ increased, too. They were positive correlation. The results showed that with the high nitrogen treatment, the nitrogen content of each organ were higher at the beginning of September, the overground grew too much and resulted in the low yield of roots. However, the sugar beet without nitrogen treatment reached the maximum value of absolute nitrogen contents, but the total nitrogen contents were too low and resulted in the fallen of leaves and low yield.

Among the treatment of different nitrogen levels, the nitrogen content of plant improved with the increasing quantity of the applied nitrogen, and both of them showed an extremely remarkable positive correlation, the correlation coefficient r = 0.995***.

The nitrogen distribution ratio of beet plant changed significantly because of the different growth stages (Table 1). More than 90% nitrogen was distributed to the overground in seedling stage and the distribution of nitrogen was mainly in overground, occupying 85%~90% of the amount of nitrogen in whole plant in the leaf fascicle formation stage, in which the nitrogen content of the root tuber is 10%~15%.

The distribution characteristic of nitrogen in plant was accordant to the nitrogenous metabolism in the beet, which was mainly in the earlier and intermediate stage of growth. The earlier and intermediate stage of beet growth was mainly the phase that the assimilative organs were formed and the period that the leaf quantity increased and the leaf area expanded most fast, so the distribution of nitrogen was mainly in the overground. According to the characteristic of the beet nitrogen metabolism, the nitrogenous fertilizer should be used at the beginning, assisting the top dressing of the earlier stage to ensure that the need for nitrogen in the earlier and intermediate stage of beet breeding, which also could avoid the rewardless growth of the leaf segment caused by excessive nitrogen absorbed in later stage

and the sugar accumulation.

After going into epacme of root tuber, the nitrogen distribution ratio of the root increased obviously, reaching about 50% in the middle of September. But for the beet with treatment of high nitrogen quantity, the nitrogen distribution ratio of the overground was still higher, so more photosynthesis continued to be used in the leaf growth and the maturation of the beet was lagged, which lead to root yield and the sugary ratio decreased. To obtain the beet of high yield and high sugar content the nitrogenous fertilizer must be applied properly both in quantity and time.

3.2 The phosphorus contents of sugar beet plant and their distribution

The three fertilizer elements have obvious interaction effect to each other, so the nitrogen level influences the phosphorus or potassium content of the beet plant and their distribution. Table 2 showed that absolute phosphorus content and the relative phosphorus content increased with the improving of the nitrogen level. The phosphorus content of the beet showed an extremely remarkable positive correlation to the quantity of nitrogen applied, the correlation coefficient r = 0.996***. The absolute phosphorus content of plant was lowest in seedling stage and increased with the development of breeding, reaching the maximum value in the epacme of root tuber, then decreased. The whole growth duration appeared a singlet curve. However, the relative content of phosphorus was highest in seedling stage, getting the lowest in harvest and the whole growth duration appeared a degression trend.

Table 2. The phosphorus contents of sugar beet plant and their distribution

Growing stages (Month.date)	Treatment	phosphorus contents in beet plants		phosphorus distribution in beet plant (%)	
		g/plant	%	leaves	roots
Seedling stage 5.30	N_0	0.43×10^{-2}	1.21	80.35	19.65
	N_{60}	0.47×10^{-2}	1.28	80.75	19.25
	N_{120}	0.54×10^{-2}	1.42	81.22	18.78
	N_{180}	0.56×10^{-2}	1.45	82.22	17.78
	N_{240}	0.60×10^{-2}	1.47	82.57	17.43
Leaf fascicle formation stage 7.4	N_0	0.38	1.03	78.72	21.28
	N_{60}	0.47	1.06	78.94	21.06
	N_{120}	0.52	1.14	79.85	20.16
	N_{180}	0.56	1.19	80.14	19.86
	N_{240}	0.72	1.25	80.61	19.39
Epacme of root tuber 8.15	N_0	0.99	0.73	69.47	30.53
	N_{60}	1.11	0.78	70.42	29.58
	N_{120}	1.49	0.80	71.22	28.78
	N_{180}	1.76	0.87	72.20	27.30
	N_{240}	1.99	0.93	74.08	25.92
Sugar accumulation stage 9.3	N_0	0.99	0.56	63.96	36.04
	N_{60}	1.06	0.57	64.13	35.87
	N_{120}	1.19	0.60	64.56	35.44
	N_{180}	1.36	0.63	64.99	35.01
	N_{240}	1.42	0.66	65.35	34.65

During the seedling phase of beet, 80% phosphorus in the plant was distributed to the overground part (Table 2). From the phyllome forms stage to middle August, the content of phosphorus in the phyllome accounted for a biggish proportion constantly, 70-80% of that in a plant. And in middle August, the accumulating quantity reached the peak. The accumulating quantity of phosphorus in the root

increased gradually with the growing process. After middle August, the distributing rate to the root of each kind of disposed phosphorus increased to 34-36%. Meanwhile, a great deal of sugar accumulated in the root. The distributing law of phosphorus was that at the early growing period, phyllome got the priority, cooperating firmly with nitrogen metabolism to promote the growth of the overground part. At the middle and late period, the distributing rate to the root increased obviously, which was helpful to the root development and the sugar accumulation. After middle August, the absorption of phosphorus waned. Therefore, the quantity of phosphorus supplied in the early period influenced the phosphorus level in the late period directly, thereby, related to the beet and sugar output. So in order to assure there is enough phosphorus to be absorbed during the early and middle phase and to meet the requirement in the whole growing period, the use of fertilizers in beet planting should mainly depends on the basic ones, with the seed fertilizers to be auxiliary.

3.3 The potassium contents of sugar beet plant and their distribution

The content of potassium in beet plant also increased with the enhancement of the nitrogen level (Table 3). They were significantly positive correlation, the correlation coefficient r = 0.996. The absolute potassium content was the lowest in seeding stage and reached the maximum at epacme of root tuber. The relative content of potassium was highest in seedling stage and lowest in harvest time.

Table 3. The potassium contents of sugar beet plant and their distribution

Growing stages (Month.date)	Treatment	potassium contents in beet plants		potassium distribution in beet plant (%)	
		g/plant	%	leaves	roots
Seedling stage 5.30	N_0	1.72	4.76	91.08	8.92
	N_{60}	1.81	4.88	91.23	8.77
	N_{120}	1.87	4.91	91.31	8.69
	N_{180}	1.93	4.95	91.45	8.55
	N_{240}	2.10	5.13	91.53	8.47
Leaf fascicle formation stage 7.4	N_0	0.69	1.89	80.32	19.68
	N_{60}	0.91	2.06	81.38	18.62
	N_{120}	1.01	2.23	82.32	17.68
	N_{180}	1.08	2.28	82.53	17.47
	N_{240}	1.37	2.39	82.63	17.37
Epacme of root tuber 8.15	N_0	2.22	1.63	77.05	22.95
	N_{60}	2.59	1.68	77.74	22.26
	N_{120}	3.28	1.76	78.52	21.48
	N_{180}	3.63	1.79	78.64	21.36
	N_{240}	3.95	1.85	78.89	21.12
Sugar accumulation stage 9.3	N_0	1.94	1.09	71.83	28.17
	N_{60}	2.10	1.13	72.25	27.75
	N_{120}	2.36	1.19	73.08	26.92
	N_{180}	2.50	1.19	73.44	26.56
	N_{240}	2.62	1.22	73.67	26.33

The distribution situation varied at different growing stages. 90% potassium was distributed to leaves and 10% was in roots (Table 3). The distribution of potassium to leaves was quite high in leaf fascicle formation stage and epacme of root tuber, which was 80%. The potassium in roots was increased with the proceeding of growing stages, which was 28% when harvesting.

The distribution regularity of potassium was mainly on leaves at the beginning of growing,

cooperated tightly with nitrogen and phosphorus metabolism and promoted the formation of assimilation organs. Later, the distribution of potassium obviously increased in roots, which could be benefits for the growing of roots and accumulation of sugar.

4. Conclusions

4.1 The content of nitrogen in beet plant increased with the elevation of nitrogen amount, which showed significantly positive correlation; The absolute content of nitrogen increased gradually with the growing proceeding, and reached the peak in root formation, then decreased.

4.2 The nitrogen level influenced the absorption of phosphorus and potassium by beet plant. The content of phosphorus and potassium showed significantly positive correlation with nitrogen amount used. Nitrogen showed obvious interaction effect with phosphorus and potassium.

4.3 Nitrogen fertilizer should be applied as basic fertilizer, then as accompanied fertilizer, phosphorus and potassium fertilizers should be used as basic ones.

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Correction:

The paper named "*Study on the Preparation and Regeneration of Protoplast from Taxol-producing Fungus Nodulisporium sylviforme*." which has been published in "*Nature and Science*"[2004,2(2): 52-59] should be corrected in page 58 as follows.

Original (page 58)	Correction
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Discussion on Intellectual Property's Private Right Nature

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Abstract: Intellectual property is a kind of private right. It is neither equal to right of public nor to civil right, but it has the factors of both right of public and civil right. It is very necessary to take intellectual property as a kind of private right legal protection. Under the situation of conflict of powers, it needs to fully consider the coincidence of intellectual property and the other two rights. [Nature and Science. 2004;2(4):84-86].

Keywords: intellectual property; private right; right of public; civil right; coincidence

1. Concept of intellectual property

Intellectual property is also called intangible property. From the point of the source of the proprietary right of intellectual property, it is a kind of private right that regulates the property ownership of intellectual products. Most countries' juristical works, laws and even international treaty all define the concept of intellectual property from the point of extension. At present, there are the following three kinds of influential definitions in the world:

1.1 The Establishing World Intellectual Property Organization (WIPO) Convention issued on July of 1967 stipulate, the intellectual property includes: (1) literary, artistic and scientific works; (2) performances of performing artists, phonograms, and broadcasts; (3) inventions in all fields of human endeavor; (4) scientific discoveries; (5) industrial designs; (6) trademarks, service marks, and commercial names and designations; (7) protection against unfair competition; (8) all other rights resulting from intellectual activity in the industrial, scientific, literary or artistic fields. Up to March 10, 2004, the state members of WIPO Convention have been 180 countries (China took part in the organization in 1980). Because the convention has the regulation in Clause 16 that for this convention there should not be any retention, we can think that, most of the countries in the world have accepted the above definitions about intellectual property.

1.2 In the *Trade Related Intellectual Property Statement* (TRIPS) of World Trade Organization (WTO), the first clause of the first part define the scope of the intellectual property included in the statement: (1) copyright and related rights; (2) trademarks; (3) geographical indications; (4) industrial designs; (5) patents; (6) layout-designs (topolographies) of integrated circuits; (7) protection of undisclosed information.

1.3 1992 Tokyo meeting of Association of Industrial Property Protection (AIPPI) thought that intellectual property can be divided into creative results rights and identifying marks right two classes. In which, the first class includes 7 items, those are patent for invention right, integrated circuits, plant varieties, know-how right (also called technical know-how right), industrial designs, copyright and software right. The later class includes 3 items, those are trademarks, commercial name right (also called trade name right) and other identifying mark right relating with prohibiting unfair competition.

2. Legal character analysis of intellectual property

The preamble principle of the TRIPS points out explicitly that the intellectual property is private right and this makes a basic orientation of the legal character of the intellectual property. Both the intellectual property and the civil right are private right and the two have very close connection. However, because the counter-performance thing of civil right is corporeal thing, the counter-performance of intellectual property is intangible thing and the continuous development of the intellectual property legal institution, the difference between the two becomes more obvious day by day, can not take intellectual property as

equal to civil right simply and should treat them separately. To be specific, intellectual property and civil right have the following differences:

(1) From the point of the social relationship that the two adjust, the social relationship that the intellectual property adjusts is not the social relationship among subjects with equal status, for example the relation between the organization for examination and approval of patent and the patent applicant, the relation between patent administrative authority and patentee and the relation between applicant of trademark registration and trademark examination organization. Besides, the administrative management relationship existing a lot in the intellectual property field makes the intellectual property can not be classified into the category of civil right.

(2) From the point of the contents of the two rights, the intellectual products as useful information combination, have a very special attribute, that once they are published, they will become public products called by economics, everyone can get it and use; while the users usually will not pay consciously for their use action. This makes the creator of the intellectual products and related input cannot get counter repayment. Because the intellectual property is intangible, the occupancy of the owner of the intellectual products to the chose is a kind of virtual occupancy, the producer of the intellectual products cannot invoke the traditional property institution in civil right to claim exclusive right of use to his chose.

Compare with other rights of real right in civil law, the difference between the two is obvious. First, intellectual property is the right of control to intangible thing. Second, intellectual property can have many real rights on one thing, for example the patentee enjoys the rights of prohibiting production, use, promise selling and importing, etc. to his invention creation. Although intellectual property and civil right are both rights against the entire world, the civil right's right against the entire world is absolute. Within the scope of legal prescription, it can exclude all people's disturbance, while the intellectual property's right against the entire world is relative. It is often restricted by other obliges including real right obliges.

(3) From the point of the acquisition and limitation of the right, there are two ways to achieve proprietary right in civil right: original acquisition and limitation acquisition. The acquisition of the intellectual property needs to go pass certain procedure for appeal, confer exclusive right after the state agency checking it as qualification; while the acquisition of copyright that gets legal protection automatically has no much difference to the original acquisition in civil right; besides, from the point of limitation, the real right in civil law is sine die effective, the effectiveness of real right is correspondent to thing's effectiveness, the effectiveness of intellectual has expiry limitation.

(4) From the point of the territorial scope of the rights that are protected by the laws, territorial protection is an important feature of intellectual property protection, the protective efficiency of the intellectual property cannot exceed the country's territorial scope, while the real right is protected by the lex loci site of international private law. No matter where the thing goes, the obligation can claim to the ownership right of the thing.

3. Intellectual property as the juristical basis of the private right protection

The right basis of the intellectual property can be traced back to the lex natural of Roman Law. It has both the characteristics of property right and right of personality, and its philosophical basis is equality. This is giving out labor that should get correspondent repayment. As the development of laws, the intellectual property theory also goes through a developing stage from natural right to social contract theory. At the end of 20th century, the right standard in civil right transfers to obligation standard. Influenced by this, from one side, the intellectual property law protects private property right. From another side, it also takes promoting advance of science and technology and the country's economic development, and the highest object. The first clause of Chinese current patent law prescribes explicitly that, to protect invention-creation patent right, encourage invention and creation, be benefit for application and dissemination of invention and creation, promote advance and creation of science and technology and adapt for demand of social modernization drive to draft this law.

Establish efficient private property law

institution can encourage people to create and develop economy. One of Noble prize winners Douglas Nose thought that one efficient property institution that includes encouraging creation and providing adequate personal stimuli is a decisive factor for promoting economic growth. He pointed out that industrial revolution is not the reason for the economic growth of the world and the key of economic growth is institution factor, especially the institution of establishing property ownership. So, it is demanded to establish efficient property rights institution, make individual's rate of return nearly equal to the society's rate of return, stimulate and promote people to be engaged in the activities that fit for the social demand. From the point of protecting private rights, many countries, including China, have established private right protection law institution - intellectual property law institution.

4. Coincidence of intellectual property and public right and civil right

4.1 Coincidence of intellectual property and public right

Public right is the right established for protecting the State and public interests, while private right is the right established for protecting private interests. The division between public law and private law functions a lot for preventing public right interferes excessively in the private right, maintaining the sacredness of private right. However, as the law's socialization goes deep further, State's interests and individual's interests, public right and private right have many coincidence, so there are the tendencies that public law becomes as private law and public law becomes as public law. Intellectual property law is a kind of law that the tendency that private law becomes public law is relatively obvious. Intellectual property is private right and intellectual property law is thought as private law traditionally. The protecting objects of intellectual property, such as invention, works, etc., not only concerns the obligee's private interests, but also connects close with public interests, some inventions even concerns the historic progress of the whole human being's civilization. Besides, from the contents of the social relations that the intellectual property law adjust, intellectual property laws not only regulate the intellectual property obligee's private law relation because of the intellectual creation activity, but also regulate the executive command relation between State agency and intellectual property oblige. Besides the clauses that limit to the rights of the intellectual property owner which exists a lot in the intellectual property law reflect the interfere of State's public right to the private right, this reflects coincidence of these public right and private right.

4.2 Coincidence of intellectual property and civil right

Relative to intellectual property, civil right has relatively strong absoluteness. However, the absoluteness of civil real right, under the influence of the big environment of socialization of the law, also increases the content of socialization gradually. The principle of the absoluteness of real right started from Roman Law times. Individual's freedom is the highest ideal at that time, ownership right is indispensable tool by which individuals realize free personality, and the laws made absolute protection to it. However, as the development of capitalistic economy day by day, principle of real right absoluteness disposed its disadvantages gradually. At the end of 19th century and the beginning of 20th century, a new law thought appeared, that is the socialization of ownership thought. This thought thought that, on the basis of human nature, ownership should be mastered and owned by individual. But when the individuals exercise ownership, it must conform to the State and the society's public interests. On this point, the socialization of the corporeal property right and the intangible property right becomes public right have some coincidence in some aspects.

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The Relationship Between Ownership Structure and Firm Performance: an Empirical Analysis over Heilongjiang Listed Companies

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Abstract: This paper investigates whether ownership structure has significant effects on the performance of listed companies in Heilongjiang Province, and in what ways it does. We will use the descriptive and empirical method to analysis the relationship between ownership structure and corporation performance. The studies also show the efficiency of corporation governance and its effect on performance of Heilongjiang listed-companies. Ownership structure has two implications: structure of ownership and ownership concentration. [Nature and Science. 2004;2(4):87-90].

Key Words: ownership structure; firm performance; corporation governance; ownership concentration

1. Introduction

The number of listed companies, trading volume, and total market capitalization has increased drastically since the opening of the two exchanges of Shanghai and Shenzhen of China. Shares are classified as domestic (A share) and foreign share (B share) by holder's residency. There are four categories of A shares. The state share, the legal person shares, the employee share, and the tradable. A share mostly held by individuals. The state shares are those held by the central government, local governments or solely government-owned enterprise. State shares are not allowed for trading at the two exchanges, but transferable to domestic institutions, upon approval of China Securities Regulatory Commission (CSRC). In many of the public-trade corporations, the state is the largest or majority shareholder. The legal person shares are the shares owned by domestic institutions as stock companies and non-bank financial institutions. Like state shares, legal person shares are not tradable at the two exchanges, but can be transferred to domestic institutions upon approval from CSRS. The employee shares are offered to worker and managers of a listed company, usually at a substantial discount. Employee shares are registered under the title of the labor union of the company, which also represents shareholding employees to exercise their rights. After a holding period of 6 to 12 months, the company may file with CSRC for allowing its employees to sell the shares in the open markets.

A typical listed Chinese stock company has mixed ownership structure with state, legal persons and domestic individual investors as the three predominate group of shareholders. China listed companies have a special ownership structure. State shares and legal or person shares are not permitted trading on the market. According to the research of Duyajun and Zhouyaping (2004), at the end of 2001, among 140 china listed companies, 63.5% shares were held by the top 10 share holders. Such high share holdings, will influence the internal governance and external supervision, and form "internal governance". The over control of top owners may bring financial and operate risk. So the benefit of ownership concentration will be deducted by the drawback of itself.

Prior studies show that ownership structure has relations with corporation governance, which is the key issue of state-owned enterprise (SOE) reform. Since Heilongjiang Province of China has higher percent of SOE among its enterprises, state shareholders play an important role on corporation governance of Heilongjiang listed company. To study the effects of ownership structure on performance, will help us to know the potential problems in performance and ownership structure.

2. Theoretical background and prior empirical work

Ownership structure has two implications:

structure of ownership (share percents of state, legal or institution, domestic individual holders) and ownership concentration (share percents of top five or 10 holders).

The relationship between ownership structure and corporation performance is one that has received considerable attention in the finance literature .The typical achievement among ownership structure and firm performance researches are the results of Jensen and Meckling. They divided shareholders into internal (investors with management right) and external shareholders (investors without ballot right). The conclusion of their research is value of firm depends on the internal shareholder's share, which is called ownership structure. Theoretically, more internal shareholder's share more firm value. The research also definite firm value as a function of ownership structure. Because ownership structure has links with corporate governance, it can have both positive and negative effects on corporation governance. The existing of top stockholders can give promotion to firm operating to some extends. According to the Shleifer's research, in the enterprise where corporate governance lacking protection to external investors, there is a trend that ownership became concentrate, which is a natural reaction of extend investors to protect their own benefit. Thus we can assume that at the circumstances corporation governance lacking well protection to external stockholders benefit, the firm performance will get better with the first top holders shares holdings increasing.

Empirical studies of Chinese scholars develop dramatically. Results from prior studies show that ownership structure (both the mix and concentration) has significant effects on the performance of stock companies. First, performance has negative correlations with state shares and positive correlations with legal shares. Typical researchers are Xiaonian Xu (1997) and Yan Wang. Second, the effect of ownership concentration is stronger for companies dominated by legal person shareholders than for those dominated by state. Such as Du Yajun (2003). Third, different shareholders play different effects on corporation governance. Chen Xiao (2000) showed that firm's profitability was positively correlated with the fraction of legal person shares, but it was either negatively correlated or uncorrelated with the fraction of state shares and tradable A-shares.

3. Materials and Method

Pooled data are used for Heilongjiang listed companies at year 2003. There were 33 enterprises issued shares on Shanghai and Shenzhen stock exchanges located in Heilongjiang Province by the end of 2003. For the financial position, 4 ST companies were among them.

Accounting ratios to measure firm's performance in the prior literatures are Tobin's Q, ROE and ROA. Tobin's Q is a market value of equity divided by the replacement cost of all assets, and it has been used as a major indicator of firm's performance. Since few of Chinese stock companies issues debt securities, it is impossible to estimate the market value of the companies' debt. We employed ROE (average weighted) as the performance indicator. Variables are structure of ownership (share percents of state, legal or institution, domestic individual holders), and ownership concentration (share percents of the top five holders). To reduce the possibility of spurious results caused by correlation among these variables, we included two control measures DAR—capital structure ratio, and InAssets (firm scale indicator). In the top 10 shareholders if state shares exceed the total of legal, traded A shares and others, we determined the characteristic of the company that is SOE. On the contrary the company is legal or person dominated.

Estimate equation to test the simples is $ROE=ß_0+ ß_1CO+ ß_2CR+ ß_3DAR+ ß_4InASSETS+ é$. Let ROE (average weighted) is Performance variables, and CO is ownership structure. If the firm's is SOE we state 1, otherwise 0 as the legal or person dominated firms. Let ownership concentration rations as CR, which is the share percent of TOP5 shareholders. Control variables are DAR (Total liabilities/Total Assets), and InAssets. We use SPSS software and get the results.

4. Results and Analysis

Empirical Statistics are shown as Tables 1 to

Table 1. Correlations

		ROE(W)	SOE	TOP5	InAssets	DAR
ROE(W)	Pearson Correlation	1.000	.300	-222	.378	*-.608
	Sig. (2-tailed)	.	.090	.215	.030	.000
	N	33	33	33	33	33
SOE	Pearson Correlation	.300	1.000	.588	**-.008	-.141
	Sig. (2-tailed)	.090	.	.000	.967	.433
	N	33	33	33	33	33
TOP5	Pearson Correlation	-222	.588	*1.000	-.007	-.338
	Sig. (2-tailed)	0215	.000	.	.969	.055
	N	33	33	33	33	33
InAssets	Pearson Correlation	.378	*-.008	-.007	1.000	.002
	Sig. (2-tailed)	.030	.967	.969	.	.990
	N	33	33	33	33	33
DAR	Pearson Correlation	-.608	**-.141	-.338	.002	1.000
	Sig. (2-tailed)	.000	.433	.055	.990	.
	N	33	33	33	33	33

***: Correlation is significant at the 0.05 level (2-tailed) **: Correlation is significant at the 0.01 level (2-tailed)**

Table 2. Variables and Regression

Model	Variables Entered	Variables Removed	Method
1	**DAR**		
	InAssets		
	SOE	**.**	**Enter**
	TOP5		

a. All requested variables entered. b: Dependent Variable: ROE weight.

Table 3. Model Summary of Table4.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.761	.579	.519	.28473817

a. Predictors: (Constant), DAR, InSaaets, SOE, TOP5.

Table 5. Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.567	.535		-1.060	.298
	SOE	.439	.211	.316	2.077	.047
	TOP5	-.512	.477	-.171	-1.310	.153
	InAssets	.110	.036	.380	3.103	.004
	DAR	-1.465	.307	-.622	-4.765	.000

a. Dependent Variable: ROE(W)

Firstly, we make the correlations of variables for equation ROE=ß$_0$+ ß$_1$CO+ ß$_2$CR+ ß$_3$DAR+ ß$_4$InASSETS+ é. As Table 1 shows, there is a positive correlations between performance and ownership structure as well as the scale of Assets (InAssets). On the contrary, ownership concentration and capital structure (DAR) have negative effects on performance.

Then we run regression of performance variables. Table 3 shows R^2=*0.579*, that means the equation is

effective. The relations between ROE and every variable are obviously. As Table 5 shows, the coefficients of variables are effective except constant $ß_0$. ROE has good correlations with SOE and InAssets as the *Sig.<0.05*. Though ownership concentration *Sig. is 0.153>0.1*, it is acceptable.

The results imply some problems arise from performance, ownership structure and corporation governance in Heilongjiang listed companies. One problem is **t**his study gives an opposite result that compare with prior literatures. For listed companies in which the state owners equity, local offices or officials of local finance bureaus exercise owner's rights on behalf of the state. This may arise a series of principle-agent problems from this institutional setting in the state-controlled stock companies. Such as shareholders of government may not have sufficient incentives to preserve and increase the value of state properties. So most researches show that the performance of SOE is not better than legal or person enterprises. For this study, Heilongjiang shows an opposite results that there is a slight positive correlations between firm's performance and state shares, because of the internal and external effects. These state enterprises have operated many years before they transfer to listed stock companies, some of them have regular management and human resource, which are the factors to improve firm performance. Another reason is Heilongjiang Province have more SOE than other areas in china, its national economy is strongly depended on SOE. Due to their important role in the national economy, some SOE still under the protection of government such as they have preference purchase and sale rights, which are not owned by legal or person firms. The other problem is corporation governance in legal or person listed companies are not developed and performed very well. Shown as data, three among four ST companies in Heilongjiang are legal or person firms. Performance of most legal or person quoted companies are not good enough compare with the SOE listed firms located in developed areas in China. Internal control and management in such firms should be strengthened. The last one is a firm profitability is negatively correlated with ownership concentration which has the same result with prior literatures, but it is not very strong. The negative effect of ownership concentration suggests that an over concentrate ownership structure is not a best way to improve efficiency of the both state and legal or person stock companies. To improve performance ownership diversification is a trend in the future enterprise reform.

5. Conclusions

It emerges from the above discussion that the different forms of ownership may have implications for corporation governance and performance of firms. First, empirical evidence showed in this paper points to the efficient correlations to state ownership, there are still some government interference and protection in Heilongjiang listed companies. Second, performance of legal or person enterprise are not good enough, so ownership diversification of state shares should be taken in a long run but not immediately. Third, excepting to strength corporation governance and internal management control, legal or person listed firms should get some support and motivation from the Heilongjiang government.

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Comprehensive Evaluating Model Based on Coefficient of entropy and Grey relating Degree to Underground Water Quality

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Abstract: According to independence and non-compatibility of indexes in the evaluating of water quality, this research builds up a new model based on coefficient of entropy to evaluate water quality, which combines entropy value theory and grey relating degree together. Entropy value method is adopted to calculate coefficient of weight and this avoid problems of distribution difficulty through using experts' experience. A new method is exercised to evaluate water quality in this paper, the outcome from application shows applicability to water quality evaluating. [Nature and Science. 2004;2(4):91-94].

Key Words: grey relating degree； water quality； entropy； evaluating

1. Introduction

Evaluating water quality is an important basic work to administration and programming of water environment. There are some evaluating methods, such as integrated index method, attribute recognition method, fuzzy mathematic analysis, multi-criteria comprehensive evaluating method and ANN etc. These methods possess respective specialties. Because of independence and non-compatibility of evaluating indexes, much useful information had been lost, even errors are gained when carrying through an evaluating water quality with those methods. For instance, integrated index method has a stronger subjectivity. It is very difficult to judge which grade it belongs to when the index of water body is on the boundary between two adjacent grades; multi-criteria comprehensive evaluating method is short of reliable way to figure out the coefficient of weight. The quantity and regularity of the sample aren't restricted to grey relating degree method, furthermore with less calculation and more clear thought, it can avoid unsuitable complexion of quantitative and qualitative analysis (Hu, 2000). Simple count mean deviation, AHP method and standard deviation are usual adopted to the calculation of the coefficient of weight in the evaluating of grey relating degree, these methods can impact the outcome of calculation to a certain extent. According to the differentiation degree of indexes, the notion of information entropy is introduced to the evaluating. It can make outcome more objective and more compatible with the actual situation, and provide us with a new method to comprehensive evaluating water quality.

2. Grey relating degree model based the coefficient of entropy

2.1 Grey relating analysis

2.1.1 Ascertain analysis sequence

Considering the aggregation of decision region as A : A ={scheme 1， scheme 2， …， scheme m }= $\{A_1, A_2, \cdots, A_m\}$ and the aggregation of evaluating indexes as V : $V = \{V_1, V_2, \cdots, V_n\}$. Marking attribute value of scheme A_i to index V_j as X_{ij} ($i = 1,2,,\cdots,m; j = 1,2,\cdots,n$).

2.1.2 Structure decision-making matrix

For the sake of eliminating the distinctness degree resulted from different dimension before evaluating, it is necessary to process non-quantized dimension. Generally speaking, the index is divided into the type of benefit and the type of cost, the index so-called type of benefit means better and better as the index turn bigger, and the index so-called type of cost means better and better as it turn smaller. Given the index of comparative optimum scheme A_0 as X_{0j}, at the same time, meeting the conditions as follows, when index V_j belongs to the type of benefit, $X_{0j} = \max(X_{1j}, X_{2j}, \cdots, X_{nj})$, when index V_j belongs to the type of cost, $X_{0j} = \min(X_{1j}, X_{2j}, \cdots, X_{nj})$. Name X_{ij} :

$$X'_{ij} = X_{ij} / X_{0j} \ (i = 0,1,\cdots,m; j = 1,2,\cdots,n) \quad (1)$$

Non-dimension matrix can be gained by non-quantizing dimension disposal:

$$X'_{0j} = [1,1,\cdots,1]$$

$$X'_{ij} = [X'_{i1}, X'_{i2}, \cdots, X'_{in}] \quad (2)$$

At this time, consider the increased matrix including the comparative optimum decision scheme $X = (X_{ij})_{(m+1)\times n}, (i = 0,1,\cdots,m; j = 1,2,\cdots,n)$ as the decision-making matrix of A to V.

2.1.3 Calculate sequence of absolute difference and minimum difference, maximum difference of two poles

The sequence of absolute difference: $\Delta_{ij} = \left| x'_{ij} - x'_{0j} \right|$ (3)

The minimum difference of two poles: $\Delta(\max) = \max\max(\Delta_{ij})$ $(i = 0,1,\cdots,m; j = 1,2,\cdots,n)$

The maximum difference of two poles: $\Delta(\min) = \min\min(\Delta_{ij})$ $(i = 0,1,\cdots,m; j = 1,2,\cdots,n)$

2.1.4 Calculate the coefficient of relating degree

The coefficient of relating degree of ith scheme jth index (shows the degree of relation between the compared sequence and the criterion sequence) ξ_{ij} can be defined as:

$$\xi_{ij} = \frac{\Delta(\min) + \rho \cdot \Delta(\max)}{\Delta_{ij} + \rho \cdot \Delta(\max)} \quad (4)$$

Where, ρ is coefficient of distinguish, and its' value vary in the extent (0，1), generally speaking, its' value is given to 0.5 (Qiao, 2004; Men. 2003).

2.2 Calculate the coefficient of weight by way of entropy value method

Entropy value method is a way through which indexes' coefficient of weight can be gained with judgement matrix composed by value of indexes (Qiou, 2001; Yan, 2003), the method can avoid the subjectivity of indexes' coefficient of weight as much as possible, and make the outcome more suitable to fact. Its' steps is as follows:

(1) Structure judgment matrix including m schemes and n indexes:

$R = (x_{ij})_{mn}$ （$i = 1,2,\cdots,m; j = 1,2,\cdots,n$）

(2) Carry through normalization disposal, and get normalized judgement matrix B:

$$b_{ij} = \frac{x_{ij} - x_{\min}}{x_{\max} - x_{\min}} \quad (5)$$

Where, $x_{\max}$、$x_{\min}$ mean the most satisfactory value or the most unsatisfactory value under the same index and different schemes.

(3) According to the define of entropy, there are m schemes and n indexes, the entropy of indexes can be defined as:

$$H_j = -\frac{1}{\ln n}(\sum_{j=1}^{n} f_{ij} \ln f_{ij}) \quad i = 1,2,\cdots,m; j = 1,2,\cdots,n \quad (6)$$

$$f_{ij} = \frac{b_{ij}}{\sum_{j=1}^{n} b_{ij}}$$

In order to make $\ln f_{ij}$ a meaning, you need to assume that when $f_{ij} = 0$, $f_{ij} \ln f_{ij} = 0$, but when $f_{ij} = 1$, $\ln f_{ij}$ =0 do not suit the actual condition of, and against the meanings of entropy, so modify f_{ij} as:

$$f_{ij} = \frac{1 + b_{ij}}{\sum_{j=1}^{n}(1 + b_{ij})} \quad (7)$$

(4) Calculate the weight of entropy of indexes W:

$$\omega_j = \frac{1 - H_i}{n - \sum_{i=1}^{m} H_i}, \quad W = (\omega_j)_{1\times n}, \quad \sum_{j=1}^{n} \omega_j = 1 \quad (8)$$

2.3 Calculate the relating degree

The relating degree E_i is defined to approach degree between the evaluated schemes and the criterion scheme, the bigger its value, the more adjacent to the optimum, whereas the further from the optimum. So you can make an order on the basis of the relating degree, and classify scheme in the light of criterion value. The matrix E_i can be expressed as:

$$E_i = \begin{bmatrix} & A_1 & A_2 & \cdots & A_m \\ E_i & E_1 & E_2 & \cdots & E_m \end{bmatrix} \quad (9)$$

Where, $E_i = \sum_{j=1}^{n} \omega_j \xi_{ij} \quad (j = 1,2,\cdots,n)$

3. Case study

The case is from chemistry zone in Handan city (Shu, 1998). There are 7 survey spots in all, and every spot includes rigidity of water body (CaO), SO_4^{2-}, Cl^-, F and organic P, according to the criterion, water body can be classified as three grades, □ is not-polluted water body, □ is polluted water body, □ is severely polluted water body. The data of factual measure and criterion are presented in Table 1.

Table 1. Value of evaluating indexes of factual measure and criterion

Survey spot	Rigidity	SO_4^{2-}	Cl^-	F	Organic P
1	1289.06	192.05	1856.05	1.20	0.050
2	1804.00	153.21	2573.50	1.35	0.171
3	1176.34	277.80	2094.07	1.35	0.384
4	689.44	142.20	782.32	0.73	0.019
5	1422.32	1120.05	726.88	0.99	0.028
6	1381.55	217.02	1694.78	0.67	0.051
7	397.66	279.02	216.04	0.63	0.022
□ grade	250.00	250.00	250.00	1.00	0.050
□grade	400.00	500.00	350.00	1.50	0.100
□grade	1700.00	1000.00	1250.00	3.00	0.500

3.1 Structure the decision matrix

Make data in Table 1 dimensionless, in this case, you can know that all the indexes are the type of benefit, so can use equation (1), then structure the increased matrix X with the data and the optimum scheme:

$$X = \begin{bmatrix} & V1 & V2 & V3 & V4 & V5 \\ A0 & 1.0000 & 1.0000 & 1.0000 & 1.0000 & 1.0000 \\ A1 & 0.7146 & 0.1715 & 0.7212 & 0.4000 & 0.1000 \\ A2 & 1.0000 & 0.1368 & 1.0000 & 0.4500 & 0.3420 \\ A3 & 0.6521 & 0.2480 & 0.8137 & 0.4500 & 0.7680 \\ A4 & 0.3872 & 0.1270 & 0.3040 & 0.2433 & 0.0380 \\ A5 & 0.7884 & 1.0000 & 0.2824 & 0.3300 & 0.0560 \\ A6 & 0.7658 & 0.1938 & 0.6586 & 0.2233 & 0.1020 \\ A7 & 0.2204 & 0.2491 & 0.0839 & 0.2100 & 0.0440 \\ B1 & 0.1386 & 0.2232 & 0.0971 & 0.3333 & 0.1000 \\ B2 & 0.2217 & 0.4464 & 0.1360 & 0.5000 & 0.2000 \\ B3 & 0.9424 & 0.8928 & 0.4857 & 1.0000 & 1.0000 \end{bmatrix}$$

3.2 Structure the matrix of coefficient of relating

Structure the sequence of absolute difference on the basis of equation (3), and calculate the minimum difference and the maximum difference of two pole of every scheme, then work out the coefficient of relating by equation (4) and structure the judgment matrix of coefficient of relating ξ_{ij}:

$$\xi_{ij} = \begin{bmatrix} & V1 & V2 & V3 & V4 & V5 \\ A0 & 1.0000 & 1.0000 & 1.0000 & 1.0000 & 1.0000 \\ A1 & 0.6014 & 0.3425 & 0.6216 & 0.3970 & 0.3483 \\ A2 & 1.0000 & 0.3333 & 1.0000 & 0.4180 & 0.4223 \\ A3 & 0.5532 & 0.3647 & 0.7109 & 0.4180 & 0.6746 \\ A4 & 0.4127 & 0.3308 & 0.3969 & 0.3430 & 0.3333 \\ A5 & 0.6706 & 1.0000 & 0.3896 & 0.3709 & 0.3375 \\ A6 & 0.6478 & 0.3487 & 0.5729 & 0.3371 & 0.3488 \\ A7 & 0.3559 & 0.3560 & 0.3333 & 0.3333 & 0.3347 \\ B1 & 0.3333 & 0.3572 & 0.3366 & 0.3721 & 0.3483 \\ B2 & 0.3563 & 0.4381 & 0.3465 & 0.4413 & 0.3755 \\ B3 & 0.8820 & 0.8011 & 0.4711 & 1.0000 & 1.0000 \end{bmatrix}$$

3.3 Calculate coefficient of weight by way of entropy value method

Structure normalized judgment matrix B by using equation (5):

$$B=\begin{bmatrix} 0.6338 & 1.0000 & 0.5537 & 0.2139 & 0.7286 & 0.6996 & 0.0000 \\ 0.0510 & 0.0113 & 0.1387 & 0.0000 & 1.0000 & 0.0765 & 0.1399 \\ 0.6957 & 1.0000 & 0.7966 & 0.2402 & 0.2167 & 0.6273 & 0.0000 \\ 0.7917 & 1.0000 & 1.0000 & 0.1389 & 0.5000 & 0.0556 & 0.0000 \\ 0.0849 & 0.4164 & 1.0000 & 0.0000 & 0.0247 & 0.0877 & 0.0082 \end{bmatrix}$$

By way of equation （6）, （7） and （8） you can get:

$H_j = (1.0349,1.0421,1.0337,1.0272,1.0396)^T \quad j = (1,2,\cdots,5)$

$\omega_j = (0.1965,0.2371,0.1900,0.1533,0.2232)^T \quad j = (1,2,\cdots,5)$

3.4 Calculate the relating degree of every scheme

By way of equation (9), the matrix E_i can be presented:

E_i	*spot* 1	*spot* 2	*spot* 3	*spot* 4	*spot* 5	*spot* 6	*spot* 7	*grade I*	*grade II*	*grade III*
E_i	0.4561	0.6239	0.5449	0.3619	0.5751	0.4484	0.3456	0.3489	0.3912	0.8292

3.5 Analysis of the outcome

In the light of the relating degree that be calculated, the polluted degree of groundwater body in chemistry zone of Handan city can be make a order as: spot 2, spot 5, spot 3, spot 1, spot 6, spot 4 and spot 7. Compared with the relating degree of criterion, you can get the result: the water body of spot 2, spot 5, spot 3, spot 1 and spot 6 belong to the extent of severely polluted water body; spot 4 belongs to the polluted and spot 7 belongs to the non-polluted. The outcome so you say is consistent to that of the literature [6] and using comprehensive evaluating method (Table 2), what the calculation gain shows it is feasible and reasonable to apply this way to evaluating groundwater quality.

Table 2. Comparison of evaluating outcomes

Methods	spot1	spot2	spot3	spot4	spot5	spot6	spot7
Attribute recognition method	β	β	β	α	β	β	–
comprehensive evaluating method	β	β	β	α	β	β	α
Grey relating degree method	β	β	β	α	β	β	–

4. Conclusion

Needing to consider completely attribute of many indexes in evaluating water quality, the method and criterion of evaluating water quality is not very mature up to the present. Applying relating degree evaluating model based on the coefficient of entropy to the comprehensive evaluating water quality can effectively account for the grade and the sort of water body, and provide us with a new way to evaluate reasonably water quality. This model considers more objective information entirely and scientifically, with clear thought and convenient calculation, and comes out to be one more effective method. In addition, this method can be applied to project decision-making, bidding and evaluating sustainable utilization of regional water resources and so on.

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