Why is Captive Breeding Important?

All over the world animals are disappearing because the forest that is their home is being cut down. The animals are also hunted for food, medicinal products and for the use of their skins. This is tremendously disadvantageous for indigenous people who depend on the forest for their survival. In many places they are already gone. By breeding animals in captivity, it is possible to replace animals that have been removed from the forest. The idea is to replace animals that have been removed for the same reason that trees are replanted in areas that have been logged. The methods used for breeding the animals are also critical for success in captive breeding. Genetic management of those species that are selected to be bred is necessary for long term success in any captive breeding program.

Genetic Management of Species in Captivity

The animals must come from many different places within their normal range so that they are certain to be from different parents. These are called the "founders." The founders are carefully managed. No related animals are allowed to breed together. This management plan creates what is called a healthy "gene pool." The important point is that they are not related to each other or "inbred." The baby animals called "offspring" will later be utilized for the perpetuation of future generations.

Initially, the first generation will serve as the basis for the second breeding generation. We retain two males and two females from each pair of founders animals. The founders are then kept in production.

This method produces a healthy population of animals and prevents production of weak and crippled offspring. The creation and management of "outbred populations" of endangered species assures that healthy animals are available for restocking the forest over many generations.

The Benefits of Captive Breeding

It is important that the Captive Breeding Program be planned in coordination with the ongoing conservation plans. This would establish a genetically sound breeding population of threatened and endangered species in captivity.

2. Wild Species as a Renewable Resource

Species bred in captivity can increase their numbers faster than is possible in the wild. Certain wild species can be bred to replace depleted stock. Larger enclosures are a good idea. However, the management of small populations without genetic deterioration demands that unrelated founders be kept in pairs. The larger enclosures can be filled with pairs of various species that will not breed but can live together. For example a breeding pair of tapir can be kept together with a breeding pair of agouti (two different species if that is possible), a breeding pair of tapir, a breeding pair of peccary, of acouchy, paca, pacarana etc. The young animals must be removed before they become of breeding age. This is to prevent random breeding. (A rather lengthy appendix follows.)

APPENDIX

THE GENETIC EQUATIONS THAT APPLY

Genetics, as it applies to populations, is an odd mix of the qualitative and the quantitative. This is especially true when it applies to the genetic component of biodiversity.

> Let us think of ***islands*** and isolated areas in the forest. Those changes which occur as a consequence of selection, or are about to occur when an ecosystem is isolated, are very dynamic. This is also true of ***small founder populations***.
>
> We are often asked to look at an ecosystem and evaluate it. The single observation is an artificial slice of life, rather portrait of a subject which is susceptible to rapid change. With respect to genetics of populations, we are taught to think of gene compositions as being static, from generation to generation. However in todays' world frequently natural populations are isolated and fragmented. Or, as in the case of Fátima once populations of species are cut off from their normal patterns of migration, are isolated or are subject to selection, his stability goes out the window. At this point dynamic and far reaching processes take place. Any description of genetic diversity without taking into account those vital trends that are in operation, leads to an incomplete description.

If one merely considers numbers, isolated from processes, such a picture cannot give more than an illusion of the total biodiversity. It doesn't matter whether a catalogue is complete citing genes, species or organisms with reference to the quantitative components of an ecosystem. One must take stock of the trends and processes which are at play in order to arrive at a useful picture of the biodiversity of a system. These ongoing processes as well as qualitative differences, serve as indicators of the change. (Let us consider an example of two tropical rain forest systems which we will examine in more detail later. Los Tuxtlas has a research station in Mexico which has been studied in comparison with one, Lacandon, in Chiapas. The study showed that in a tropical rain forest, total plant biodiversity was dependent on the presence of a variety of animal life. The forest without the animals, Los Tuxtlas, contained a healthy plant population including a greater population of new shoot growth surviving. However, there were factors that had reduced the species composition of Los Tuxtlas so that its plant diversity was only a fraction of the forest diversity of Lacandon.)

Understanding Population Equilibrium

Components of Population equilibrium

The components of population genetics are essentially quite simple. The composition of any given population will tend to remain the same for generation after generation if:

a.. migration in and migration out balance;
b. mutation and selection are not factors;
c. choice of mates is random;
d. the number of individuals comprising the population is sufficiently large.

a. Migration and Emigration

Except for island populations, most of the target species of the tropical rain forest that we consider as parts of the endangered keystone guilds have been traditionally freely migrating species throughout a realm. There are some local difference between populations. Therefore in the past, we need not be concerned about migration and emigration affecting any reintroduction. However, these are the most complex factors and are completely context dependent: **Caution today we must take into account the concept of the fragmentation of habitat and the virtual island like formation of tropical rain forest that is timbered in sectors;** thus any scenarios for release that we entertain must be based on questions concerning the:

1) presence of existing relicts of remaining populations of the target species in the specific area;
2) presence or absence of competing species:
3) target or the competitor species are ***r*** or ***k*** type strategists;
4) supply of food substances is adequate;
5) degree of isolation of release area.

Only the intelligent selection of target areas will make these factors minimal. From time to time, any number of individual animals will undoubtedly leave but, largely they will go to inhabit new areas, which is basically part and parcel of a working reintroduction scheme.

b. Mutation

This is rare event with expected occurrences in the range of one case in approximately one hundred thousand. Thus mutation can generally be ignored in the Fátima program except where chance brings us a disadvantageous or advantageous gene.

c. Selection

In the wild, the selective forces involved in specifically altering the population of a species, often benefits the survival of the species. An example of this is pesticide resistance.
This is in sharp contrast to the situation in captivity. Note well. It is important to realize that

the species in an ex situ captive breeding context may not be genetically deteriorating, but may still be not undergoing the processes of selection that increase the species fitness to survive in the wild.

DANGERS OF SELECTION FOR A RARE TRAIT

In domestic breeding, selection for a rare trait that can be of importance to the husbandryman. This process has yielded, hornlessness in the polled Hereford, high butterfat production in the Jersey cow, and short legged characteristics in the beef cattle. These advances are brought about at some genetic cost. The wild animals, particularly those in captivity (or any endangered species), have a tiny gene pool,. This is unlike the case of domestic animals which have a large gene pool. Domestic animals can be considered as a ***human 'creation.'*** Therefore, they theoretically can be manipulated for an agricultural advantage. In the small genetic population of wild species in captivity selection for rare genetic traits such as those producing the white tiger, result in tragic consequences for a momentary gain and must be avoided.

Creation of Domestic animals from Wild Animals

There is a great deal of misunderstanding about the process of domestication. However, domestication is essentially a genetic process of manipulating the gene pool to fit better into some condition that benefits humanity. In the case of wild animals, domestication stems from a selective process. Examples of this are:

1) selection for maximum production of offspring;

2) selection of a desired characteristic such as size, good looks, meat production benign disposition and some aspect giving economic advantage. *(Breeding animals in this fashion* ***would*** *tend to produce a 'domesticated' version of a wild animal species. This process can be beneficial to* ***Fátima*** *but can also rapidly lead to degeneration of the breeding stock. This has been disastrously carried out by zoos and preserves in the past).*

Even more drastic and destructive are those techniques carried out by fisheries and wildlife programs. These programs take their clue from techniques used to **MAXIMIZE** the participation of a few founders. In order to do so, they will fertilize a large number of female salmon with the sperm from one male. Let us see what sort of an effect such a genetic strategy brings about.

To understand degenerative genetic processes in small populations, we first look at a simple equation which shows how much genetic diversity one can gather in a founding population if one uses the proper number of ancestors.

Equation One. **Genetic diversity is Heterozygosity (H) so $H=1-1/2Ne$ (with N_e is defined as the effective number of reproducing animals**

N_e	H
5	**.9**
6	**.92**
8	**.9375**
13	**.962**
25	**.98**
50	.99

If we looked at the same diversity but turned to pairs realizing that a pair consisting of 2 unrelated individuals is equivalent to an N_e of 2 then we see the following changes.

N_e	H	
5pr	.95	
6pr3	**.958**	
7pr.	**.964**	
8pr.	**.969**	
13 pr.	**.98**	**The CPT number an ideal of 'the most genetic diversity for the smallest cost**
21pr.	**.988**	
44pr.	.994	

(Numbers given in bold are candidates for a closed system captive breeding population. Only availability of founders and the economic realities of the program dictate the number.)

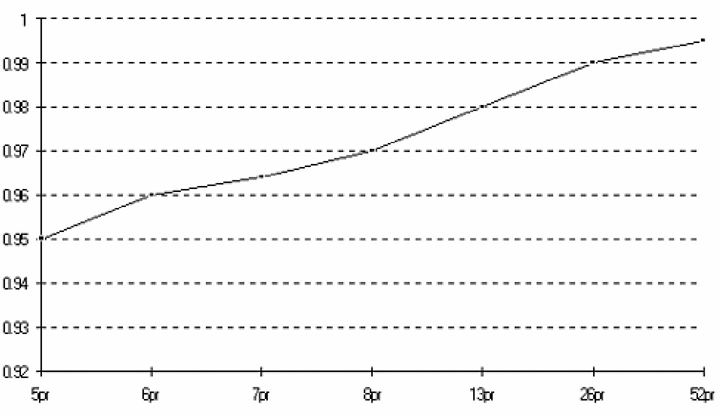


Figure One shows the implications inherent in Equation One
As we increase the founder pairs from five to fifty two, we can see that the level of H reaches 0.95 at five pairs and 0.98 by 13 pairs. It requires a doubling to 26 pairs of N_e *to gain one more H=0.01. Why we feel that the gain is important can be seen from the implications of* **Equations Three and Five**.
Using one male to inseminate many females is a traditional practice. This is only useful when the entire population is so large that the genetic health of the population is not effected. Most of the rare animals

(Rare are those whose population is under 1000) are very sensitive to genetic deterioration. When males are used in excess of females, we term this asymmetric rations. If we examine the asymmetric founder populations that are in almost universal use in tradition captive breeding programs we can see the drastic inefficiency that results.

Equation Two.. The effect of asymmetric parental sex in founders on contribution of individuals to the N_e.

The effective population is equal to four times the number of females multiplied by the males all divided by the number of females and the number of males $N_e = 4NfNm/Nf+Nm$

Thus in an ideal population the number of individuals equals the number of ***effective*** individuals.

In a population of ten, where ideally $N_e=N$, the number of males (5) will equal the females (5) and the ratio of individuals to effective individuals will be one.

Let us look at several examples **First some symmetric figures.**

males	**females**	N_e	N_e/N
7	7	14	1
13	13	26	1
if we consider the following populations of ten individuals:			
5	5	10	1

If however we let the population become asymmetric, we lose effective population size even with the same N (total number of animals

males	**females**	N_e	N_e/N
4	6	9.6	.96
3	7	8.4	.84
2	8	6.4	.64

In fact this last case gives only the same ***effective population*** size as

1	4	3.2	.64

When we become further asymmetric, we throw all our advantages of adding a greater number of founders to the population as these examples show.

1	9	3.6	.36
1	99	3.96	.0396

If we greatly increase the N of individuals but make no effort to equalize sex equations, then

males	**females**	N_e	N_e/N
1	199	3.98	.0199 (4)
1	499	3.992	.002

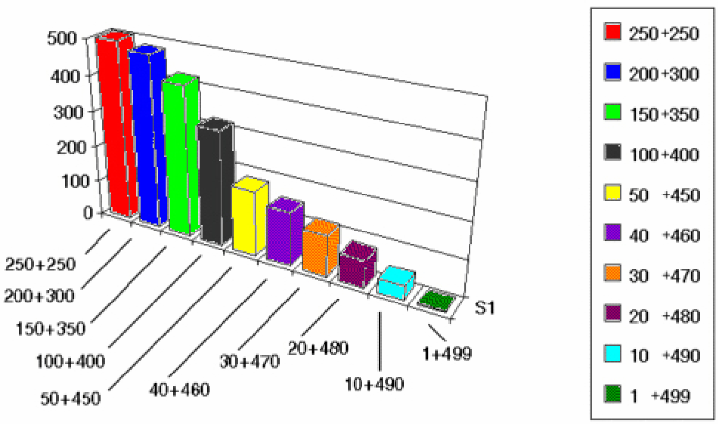
(4) (This number isn't fanciful. Fish and Game department wildlife managers have frequently used one male to as many as 200 females in the production of fry to be released.)

If we were to use the same founding population number but equalize the sex ratio we see the enormous advantage implicit in using a one to one ratio.

250	250	500	1.0

Figure Two recaps Equation Two

The N_e of several populations of 500 individuals are shown as three dimensional figure. The of sex ratios of the ten populations range from 1:1 to a final of 1:499. The effective drop in the effective population size can be seen to go from 500 down to under four, even though 500 parents are reproducing in all cases.



At CPT, we counter the threats of **domestication**, **selection** and **asymmetric founder** effects by the practices of breeding blindly and of limiting our next generation offspring from any one pair. By breeding blindly we let the computer generate the cross of any generation based on the degree of relatedness. Thus we eliminate any selection conscious or unconscious from the breeding process. We then limit the members of each next generation to two males and two females from any one pair of breeders and using these offspring equally as breeders.

d. Assortative random mating

This can be assumed if one posits that the forces of genetic drift and the founder effect must be considered. In addition, the constraints of sufficient population size and sufficient knowledge of the natural uses of male and female territories must also be taken under consideration.

e. Population Size

A captive or otherwise isolated population is only safe from genetic deterioration when it starts with a demographically sound base that contains a sufficient about of diversity (0.98 of diversity). It should fairly quickly reach 200-500 individuals. When a smaller population is to be dealt with, it is necessary to consider the dangers of genetic drift and the founder effect

When an isolated population is smaller than 100 in number, certain statistical vagaries begin to effect the composition of this population. These vagaries or factors called 'genetic drift' do not effect large populations. The way in which random genetic drift is established is primarily through the founder effect. Let us consider a hypothetical population of ten pairs. Theoretically, one would postulate that this population would consist of ten pairs of founders that are all: of approximately the same age; would all reproduce; all have the same number of offspring; and these offspring would randomly mate and continue the process. This of course is purely idealistic. There are more ways to go wrong than right. Some sires will be more sexually successful than others, some will be more prolific, some will die before achieving issue. Exactly the same considerations apply for dams. Moreover, the sex ratios of the offspring will not be the perfect 1:1 males to females. With large populations these random factors tend to cancel out. In the case of small populations, this is the process by which these populations randomly drift, from the ideal of a genetic equilibrium. The force of random drifting is very powerful and can quickly cause genetic deterioration equivalent to inbreeding and poor selection.

An equation that shows how genetic drift occurs in small populations is **Equation Three:** The rate of fixation for a single genetic trait is based on the concept sigma or standard deviation = the square root of pq/n. For our purposes sigma equals the area in 68% of the cases. 2 sigma 95% of all cases. P and Q are allelic forms of a gene we will
assume a gene frequency of 0.5 for P and Q

# founder	Sq rt pq/2n	standard deviation equals cal. dev.	relative fixation 0.5 plus or minus calculated deviation gives range of fixation
1pr .	$\frac{0.25}{4}$	.25	.25-.75
2pr	$\frac{0.25}{8}$	.176 .	34-.676

We can see that for very small populations, with very few founder individuals, the rate of fixation or loss of a common trait can occur very rapidly.
As we gradually increase the population, to the size of most captive breeding founder populations, or those populations in isolated reserves, we see that fixation is greatly slowed.

# founder	Sq rt pq/2n	standard deviation equals cal. dev.	relative fixation 0.5
5pr	$\frac{0.25}{20}$	.1118	.388-.6118

7pr	$\frac{0.25}{28}$	**.095**	**.405-.595**
13pr	$\frac{0.25}{52}$	**.069**	**.431-.569**

As we approach the safe zone in population number, we see the threat decline.

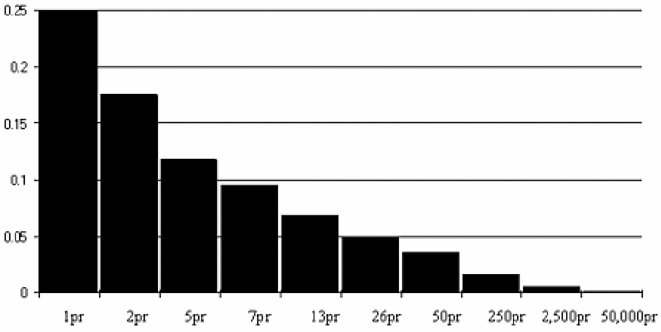
50pr	$\frac{0.25}{200}$	.035	.465-.535
250pr	$\frac{0.25}{1000}$	.0158	.484-.515

When the number goes over 500 individuals, the threat by genetic drift is negligible.

# founder	Sq rt pq/2n	standard deviation equals cal. dev.	relative fixation 0.5
2500pr	$\frac{0.25}{10,000}$	.005	.495-.505
50,000pr	$\frac{0.25}{200,000}$	.001	.499-.501

Figure Three A recapitulates **Equation Three**

The percentage rate of loss or fixation of a genetic trait per generation decreases as the number of founding pairs increases.



By the time a population reaches 250 pairs the loss is an insignificant 1.5%. The critical areas for a small gene pool is in the range of between seven and fifty pairs.

In the CPT breeding scheme, by taking 2 males and 2 females from each generation, one can reach a second generation population of 26 parental pairs. This population can be either maintained or increased.

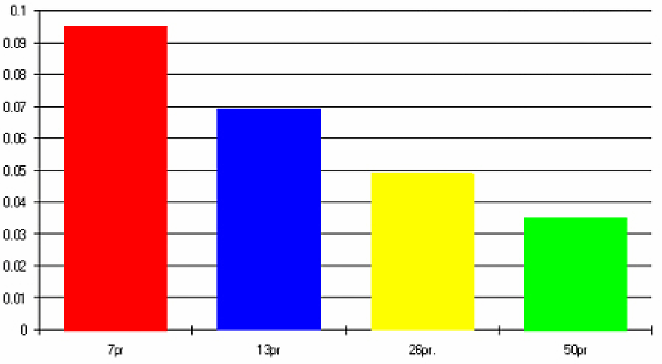
# founder	Sq rt pq/2n	standard deviation equals cal. dev.	relative fixation 0.5
26pr	$\frac{0.25}{104}$	**.049**	**.451-.549**

optional expansion beyond this gives and f2 generation of 52 pairs:

52pr	$\mathbf{\frac{0.25}{208}}$	**.034**	**.466- .534**

Figure Three A amplifies this region of **Figure Three**

It can be seen that the rate of fixation or loss falls below the significant five % level when the population reaches 26 pairs.



Therefore even though the gain through doubling yields a rather small increase in H (heterogeneity) of 98% to 99%, the rate of fixation or loss decreases to below the 5% significance level. (Another way of looking at the same phenomenon is that for this ***we gain an increased chance, greater than 42% of retaining the same proportion of any from one generation to the next.***

For any small population, this means we can come closer to the ***Hardy- Weinberg Equilibrium. The*** implications are that we must always be aware of genetic drift whenever possible.
CPT has formulated its breeding program to nullify another huge trap. **Equation four** indicates the deterioration in effective founders that can occur when family size is randomized. Here the equation which is relevant is that N_e is equal to 4 times the number of individuals in the population divided by 2 plus the variation (sigma) squared divided by

$$N_e = \frac{4n}{2 + \frac{(\mathrm{Sigma})^2}{2}}$$

If the variation is kept to only 2 individuals per family size then in CPT schema for 26 breeders for the first generation then we can see how powerful a force random litters are:

N	4N	$2 + \frac{(\mathbf{sigma})^2}{2}$	N_e	N_e/N

If families were freely allowed to vary so that they went over a variance of four then because sigma is squared the effective population ration is reduced.

26	104	$2 + (\mathbf{4})^2 = \{16\} /2 = 10$	10.4	0.4

Of course variation in family size can be much greater than four. If we look at what happens with a steady reduction of variation say if sigma is further reduced, improvement is seen.

26	104	2 + (**3**)^{2} =[9] /2 = 6.5	16	0.615

By the time sigma is reduced to two according to Crow and Kimura, N_e/N= unity.

26	104	2 +(**2**)^{2} = [4] /2 = 4	26	1.0

At this point, the population equals the effective population. However the equation shows a progression so that in a well managed population where only 2 equal sexed animals descended from each parental cross enter the breeding pool, even greater advantages can be possible.

26	104	2 + (**1**)^{2} = [1] /2 = 2.5	41.6	1.6

or if sigma becomes very small 0 to 0.1

26	104	2 + (**0.1**)^{2} = [0.01] /2 = 2.005	51.8	1995
		(**0.01**)^{2} = [0.0001] /2 -2.0001	51.997	1.9999

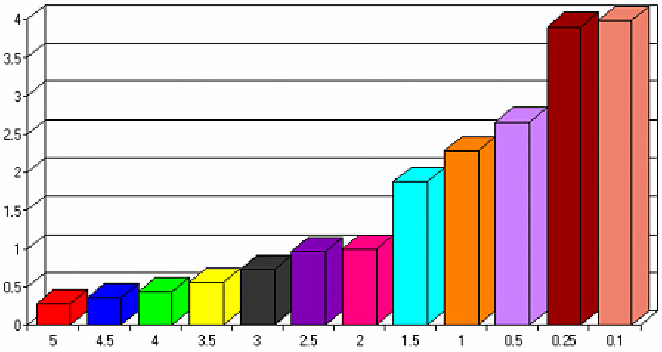
Therefore if the family size is regulated, as CPT does, then the effective founder size is more than doubled.

Figure Four A shows the implications of **Equation Four**

$$N_e = \frac{4n}{2 + \frac{(\mathbf{Sigma})^2}{2}}$$

the deterioration in effective founders that can occur when family size is randomized

An array of effective population sizes, result from having the ***variance*** in the size of families decrease from five to 0.1.



This decrease in family variance is one of the strategies that we have always maintained at the Trust. Two males and two female progeny from each set of parental founders forms the population of the next generation. This is a multipurpose schemata.

1. It becomes easier to avoid inbreeding.
2. We have seen from **Equation Two** that it avoids loss of N_e as a consequence of sexual asymmetry.
3. Equation Three shows not only the pitfall of losses in N_e resulting from family size variance but an implied gain in N_e when the variance in family size is held under two (**CPT's program holds variance symptomatic to Zero!)**

We can see the full implications of this increase in N_e by looking at the consequence of **Equation One.** We have seen in **Figure One** that:

# founder	standard deviation		relative fixation
	Sq. rt. pq/2n	equals	0.5
		calc. dev	plus or minus calculated deviation gives range of fixation
7pr	$\mathbf{\frac{0.25}{28}}$	**.095**	**.405-.595**
13pr	$\mathbf{\frac{0.25}{52}}$	**.069**	**.431-.569**
50pr	$\mathbf{\frac{0.25}{200}}$	**.035**	**.465-.535**

Now taking this into account, CPT by effectively by taking 2 males and 2 females from each generation, then one maintains a second-generation population of 26 parental pairs thus:

26pr	$\mathbf{\frac{0.25}{104}}$	**.049**	**.451-.549**

If the implications of Crow and Kimura's equation (Three) holds then each pair becomes more than doubled in value when non variable family size gives an advantage of doubling N_e.

Optional expansion beyond this gives and f2 generation of 52 pairs, thus:

13 pr now yields	0.049
26 pr	0.34

Therefore by taking two males and two females from each cross for the second generation, that effectively gives an insignificant loss through fixation of an equivalent 52 pairs with only 0.017 as rates of loss through fixation!

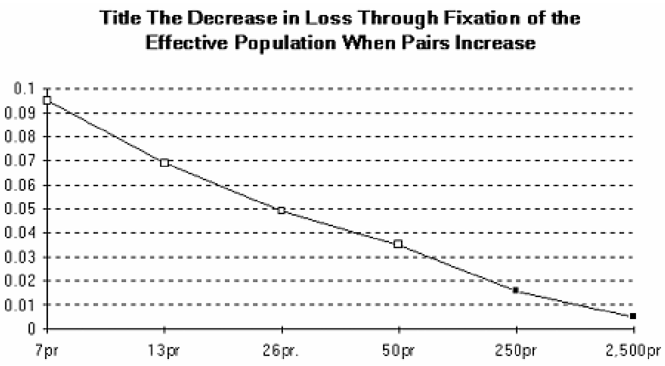


Figure Four B shows the rate of Fixation of a trait as a consequence of Equation Three

The degree of loss can be read along the x axis. In the CPT program 13 pairs have a maximum possible rate of fixation of 0.07 for one generation. Doubling the population to 26 pairs for generation two, gives a 0.049 rate. However, the theoretical advantage seen in **Figure Four A**, shows a **ratio** of increase in N_e of up to four from holding variance close to zero. This graph would imply a stability of any trait, equivalent to that found in a non regulated population of 208 individuals!

One of the consequences of dealing with a small population is the rate of loss of genetic diversity. **Equation five** governs the speed in which heterozygosity, a key measure of genetic diversity is lost in a small population. This equation is derived from that equation governing heterozygosity in a population . This equation states $H = 1 - \underline{1.}$

The rate of loss equation is derived from this by taking account of the generations **g** in the following equation

$$H = \left(1 - \frac{1.}{2 N_e}\right)^{\mathbf{g}}$$

Generations

N_e	1	2	5	6........	10	....	20
7	.93	.865	.7		.49		
13	.962	.93	.83		.68		
25	.98	.96	.90		.82		
50	.99	.98	.95		.90		

For pairs it can still be seen that despite initially high heterozygosity, long term losses develop.

N_e	1	2	5	6........	10	....	20

5pr	.95	.9025	.773	.73	.60		.36
6pr	**.958**	**.917**	**.806**	**.773**	**.68**		**.44**
7pr.	**.964**	**.92**	**.815**	**.78**	**.69**		**.46**
8pr.	**.969**	**.939**	**.85**	**.82**	**.75**		**.55**
13 pr.	**.98**	**.96**	**.90**	**.885**	**.833**		**.681**

The rate of loss is still pretty significant over a long term living time capsule. Note in CPT's schemata the effective population of 13 pairs N_e is expanded into 26 pairs in the second generation so the N_e no longer remains at 26 but instead becomes 26 pairs!

N_e	**1**	**2**	**5**	**6........**	**10**	**.........**	**20**
26	.98	.96	.90	.885	.833		.681

When the population is doubled to be 52 in the next generations loss decreases.

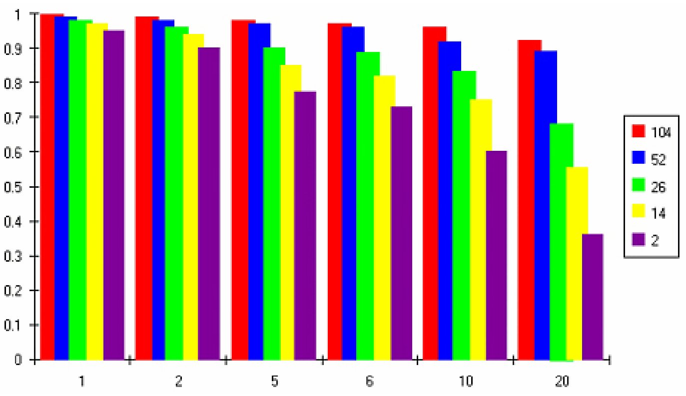
N_e	**1**	**2**	**5**	**6........**	**10**	**.........**	**20**	
52	...	.988	.97	.96	.94		.891	.

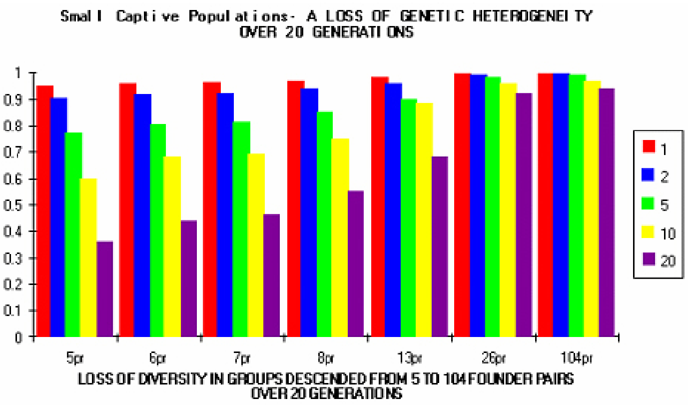
In the equations that doubled the Ne as a consequence of non variable family size,

$$N_e = \frac{4n}{2 + \frac{(\text{Sigma})^2}{2}}$$

then even less loss results for 26 pr. acts as though it started as 52 pr. and

N_e	**1**	**2**	**5**	**6........**	**10**	**.........**	**20**
	.994						
doubling for second generation	.992	.98	.97		.96		.921





Basic Genetic Strategy (a recapitulation)

It is our goal to prevent any inbreeding closer than that of third cousins, conserve the greatest number of alleles possible at the lowest cost, and to avoid any special genetic selection save that against deleterious genes.

The economic realities facing us are that most of our target species are not being maintained at all by the world's zoos so we cannot rely on them as either a source of stock or for support of any kind. The resources by which these animals can exist in truly large numbers are simply not there nor many times are their large numbers of animals available as founding stock. So instead of being able to start with hundreds of specimens of each species, the number of which will increase with each generation, we are forced to be considerably less ambitious and more realistic. Remember, in the carnivore life scheme as in human, several generations coexist at once so several hundred animals of any given species would need to constantly be maintained. The number of pairs whether seven or thirteen in the founding representing a compromise which allows the retention of more than 90% of the population heterogeneity when carried out by our plan. Which is to keep pairs separate; to keep two males and two females resulting from each cross; and to maximally out breed these animals with the most distantly related individuals of their generation to form the basis of each succeeding generation. The economic reality of this strategy is that by planning we can greatly reduce the need for a large population of animals. In CPT's scheme, 14 to 26 animals in generation I, 28 to 52 animals in generation II with members of generation III being born while some members of generation I are still alive. Following this scheme we can hope for a maximum population with approximately 50 members of a single species. To gain the next advantage in retaining a greater amount of heterogeneity we would have to start with 13 pairs. This less than 2% gain in the initial heterozygosity of a population is small. However, 13 initial pairs does provide a buffer against decay (It can be seen that 13 pairs gives 50% greater remain heterozygosity over 20 closed population captive generations). This must be balanced against the cost of population expansion. The benefit of keeping 4 individuals from each cross would give us the responsibility of housing 150 or more members of a single species.