## 1.2    What, if anything, is a Wolf?

*Raymond Coppinger, Lee Spector and Lynn Miller*

<mark>EDITORS WILL ADD WOLF DRAWING AT HIGH RESOLUTION HERE</mark>

*"In short, we will have to treat species in the same manner as those naturalists treat genera, who admit that genera are merely artificial combinations made for convenience. This may not be a cheering prospect; but we shall at least be freed from the vain search for the undiscovered and the undiscoverable essence of the term species."*
Charles Darwin (1859, 1903)

### Introduction

Species can be considered as moving targets. In every generation, there should be an adaptive response of a population of animals to an inconstant environment. This biological perspective, which is the core of the theory that has unified biology ever since Darwin proposed natural selection, is sometimes forgotten in the face of practical management decisions. For example, in the U.S. the federal Endangered Species Act (ESA) provides protection for species, subspecies, and populations. This protection, preservation, and restoration of a species, subspecies or populations could imply that the essence (*sensu* Aristotle, Linnaeus) of the species is conserved. However, it should not imply that the phenotype or genotype is eternally fixed.

Management problems in restoration programs quite often centre on the genetic purity of the species being conserved. In North America, red wolves (*Canis rufus*) to be released from captivity into the wild are thought to be hybrids of gray wolves (*Canis lupus*) and/or coyotes (*Canis latrans*) (Wayne & Jenks 1991). Even though the restorers believe their animal has the "essence" of red wolf, geneticists say the species is not pure, or is a hybrid (O'Brien & Mayr 1991). The ESA does not provide protection for hybrids—even hybrids of severely endangered species. However, such ESA principle is not applied in the red wolf case. In other cases, some opponents of restoration plans object that the animals, which were reintroduced, are not the original subspecies or population and should therefore be removed.

For those of us interested in the conservation and restoration of wild canids there are several problems illustrated in these examples. The first is that definitions of species, subspecies, populations, and hybrids are unclear (O'Brien & Mayr 1991). Not only are species moving targets, but the definitions of species are ephemeral. The second and perhaps major problem is that we do not have a satisfactory methodology for identifying species, subspecies, and populations. Taxonomists do not agree with each other, and morphometric techniques do not agree with the genetic evidence. Thus, attempting to provide species, subspecies or populations with legal definitions that can be used to define conservation plans becomes overwhelmingly difficult and often contentious.

At the simplest level one wants to preserve an endangered species because it is perceived to have intrinsic value. At a second level, there is the effort to preserve the

ecosystem. It is the ecosystem that has an intrinsic value and the various individual organisms behave synergistically to create that whole. At a third level, the attempt is the preservation of biodiversity, to maximize biodiversity as is elegantly laid out by Wilson (1999). Important to our discussion here "species and subspecies" are the units of measurement of biodiversity. A world with two species is more complex, more "whole" than a world with one species. In each of these three conservation goals, there is the underlying assumption of the fixity of species. There is a number of species now, and our job as wildlife managers is to discover, describe, and preserve as many of them as possible.

We often practice wildlife management or endangered species management as if species are a fixed value and if they were clearly separate entities. In the red wolf recovery program, animals to be released into the wild have been genetically fingerprinted with the aim of testing subsequent generations and removing animals that show signs of hybridization. In preparation for their release, the environment was cleared of other members of the genus *Canis* in order to prevent hybridization. In the case of the Ethiopian wolf, which interbreeds with domestic dogs (*Canis familiaris* or *Canis lupus familiaris* [see below]; Wayne & Gottelli 1997), the proposal is to kill or sterilize the larger population of dogs in order to preserve the endangered canid species (Laurenson et al. 1997). Thus, it appears that the belief of the wildlife managers in both projects is to regard only the endangered species as having intrinsic and important value and the common species are considered as just vermin to be removed. In both of these cases it is the assumed fixed nature of a species, and a preeminent loss of biodiversity that drives the management plan. There is no consideration that dogs or coyotes may be the more highly evolved forms or that hybridization between these "species" might be the source of the variability necessary for red wolf or Ethiopian wolves to evolve and to adapt to the changing ecosystem (Reyer 2008).

The next question is: who is qualified to define these animals as an endangered species? Are the managers of so-called endangered species free to choose among the various taxonomic systems and pick one of the many species definitions available? For example, why should wildlife managers believe the geneticists who claim that red wolves are hybrids? Have we studied their methodologies and their definitions? Have they thought out the implications of what they are saying? Even if morphometricians or geneticists could reliably determine hybridization, who says that the new hybrid essence is not good enough or does not perform adequately in the ecosystem, or reduces biodiversity?

### *Difficulties in Species Identification*

*"...while we must make do with the terminologies of Aristotle and Linnaeus, we need not take them too seriously."*
Haldane 1956

Many years ago, palaeontologist Albert Wood (1957) wrote a paper entitled: "What, if anything, is a rabbit?" Rabbits are lagomorphs. A debate ensued among famous palaeontologists (e.g., Albert Wood, George Gaylord Simpson) about whether rabbits were related to rodents. "Related" could mean they were descended from rodents, or that

rodents and lagomorphs are descended from a common ancestor, or perhaps that rabbits were the ancestors of the rodents.

The 'rabbit debate' exemplifies how experts may shift back and forth, changing their minds about ancestries—based on new evidence, and sometimes upon re-analysis of existing data. In the last fifty years, molecular biology has steadily grown in technique, and enabled new insights to many evolutionary questions. For example, Graur et al. (1996) demonstrated that rabbits were descendants of primates. It is the kind of discovery that could not have been made through morphometric analysis alone (e.g. comparison of skulls and teeth), since such phenotypic characteristics might be confounded by convergent evolution.

Having a good definition of species and other taxonomic groups and their evolutionary history is necessary for management policy. In fact, without a proper well-defined name, wildlife law as it applies to endangered species becomes impossible.

Watch a 20 kg wild *Canis* moving across a field in New England or Quebec and ask the experts, "What is it?" and you could get several answers. Some wildlife biologists still call it a coydog (a supposed hybrid between a coyote and domestic dog; Silver & Silver 1969); others think it is a subspecies of coyote (*C. latrans* var.; Lawrence & Bossert 1967; Lawrence & Bossert 1969), while still others think it might be an Algonquin wolf (*C. lupus lycaon*). And maybe it is *C. lycaon* and more closely related to the red wolf (*C. rufus*) than it is to the gray wolf (*C. lupus*; Wilson et al. 2000; Kyle et al. 2006). Still others have argued that it might be a hybrid between the gray wolf (*C. lupus*) and the coyote (*C. latrans*; Lehman et al. 1991). A similar confusion can also be noticed for canids living in the Great Lakes regions of Canada and the U.S. (Leonard & Wayne 2007, Wheeldon & White 2008).

An animal's nomenclature is critically important in the twenty-first century because, for example, if the animal is a gray wolf (*C. lupus*) or red wolf (*C. rufus*) you cannot shoot it in the U.S. because they are on the endangered species list. If it is *C. lupus lycaon*, you can shoot it in Quebec, Canada but not in the northeastern U.S. because technically it is a gray wolf. However, if it is *C. lycaon* it is unclear what the rules are in the northeastern U.S. In addition, if it is a coyote, you can shoot it. If it is any of the canids listed above or even a coydog, you cannot capture and keep it in Connecticut because it is considered a wild animal and a permit is required. However, in Massachusetts anything with dog in it is considered a dog and you can cage it. (Ironically, since the domestic dog is technically *Canis lupus fam.* (i.e. a subspecies of wolf), one could make the case that dogs be covered under the ESA. Can a species be endangered and its subspecies not?) But why is it so hard to identify our animal and why do so many people disagree?

Every species of the genus *Canis* has similar morphological features and it is somehow difficult to tell the differences among them. All Canis species are karyotypically identical, that is, all have identical chromosome numbers, sizes, shapes, and even banding patterns (Todd 1970; Chiarelli 1975; Wayne et al. 1987). All members of the *Canis* genus are inter-fertile. Hybrids are easily produced in the laboratory for study. In addition, there are growing numbers of wolf/dog hybrids and dog/jackal hybrids kept for pets. Finally, coyotes, wolves, dogs, and jackals hybridize "naturally." (Lehman et al. 1991; Vilà et al. 1997). In fact, the only barriers to reproduction among all these

species are size differences, social organizations that tend to restrict reproductive access, and of course, geographic isolation.

### *Morphometric Solutions to the Species Problem*

*"Taxonomy is written by taxonomists for taxonomists;... It is the most subjective branch of any biological discipline and in many ways is more of an art than a science."*
Cowan 1971

Traditionally, as with the rabbit/rodent problem, differentiation was based on measurement. The underlying assumption is that statistical differences in phenotype may also reflect differences in genotype. The larger the differences in phenotype, the more distantly related are the two forms.

It is an assumption however, that phenotype represents genotype. When that assumption is used to determine species as sexually isolated populations, it is often inadequate to the task. For example, dogs probably have the greatest morphological deviations (breeds) in the mammal world (Sutter et al. 2007). However, dog breeds are not distantly related one to another, and are not different species one from another. In dogs, very minor changes in onsets and offsets and allometric growth ratios can result in enormous differences in the resulting head shape. Similarly, very small allelic differences among dog breeds allow for large differences in size between breeds (Sutter et al. 2007).

Schneider (unpublished data; Coppinger & Schneider 1995) compares the skulls from five "species" of *Canis* and a number of dog breeds for differences in shape. Skull shapes were measured electronically for size-independent differences. If these two charts based on dorsal and ventral views (Fig. 1.2.1) are interpreted as phylogenetic trees, one sees immediately the problem with using morphometrics. The most diverse shapes are represented by breeds of dogs. Species such as coyotes, golden jackals, and side-striped jackals, which live continents apart, have almost identical head shapes, but change relationships dramatically depending on whether one is looking at the dorsal or ventral view. Wolves also change relationships depending on the view. In canids, differences in head shape may have little relationship to phylogeny and one cannot infer genetic relatedness nor construct a phylogenetic tree using morphometrics only.
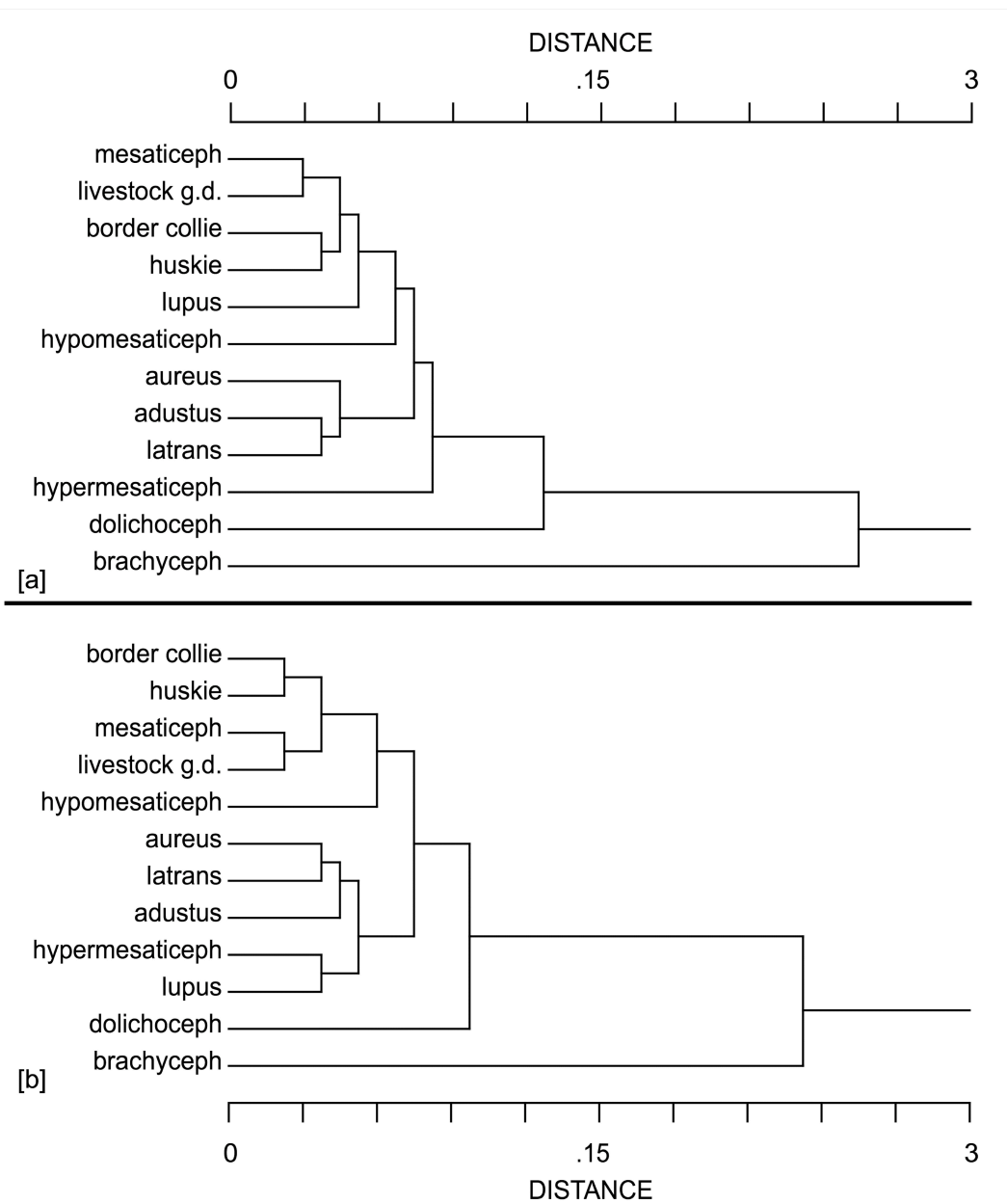
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Figure 1.2.1. Cladograms of five species of the genus *Canis*, also including assorted breeds of dogs, based on (a) dorsal and (b) ventral morphometric views of adult skulls.

### *Genetic Solutions to the Species Problem*

In the past thirty years, molecular geneticists have entered the species debate. Their intent is to find DNA sequences that will show relationships between species, and finally to apply coalescent methods to date the origins of those species (Avise 2000). If speciation is the gradual shift of allelic frequency over time, it is assumed that populations with similar allelic frequencies are more closely related, while those with radically different allelic frequencies are more distantly related. The larger those genetic differences are, the longer the time since the two species diverged.

The geneticist argument starts with the neutral mutation theory (Kimura 1968). The neutral mutation theory is based on the observation that there are genetic mutations which are neutral to selective processes; they are not deleterious nor are they advantageous to the organisms that inherit them. For example, there are mutations that appear not to affect any changes in phenotype. The neutral mutation hypothesis predicts that these random mutations do not affect fitness, thus they will not be selected against, or for, and therefore will accumulate over time. Leonard et al. (2002) and Savolainen et al. (2002) have applied such an approach to the problem of dog origin from wolves.

The flaw in this reasoning derives from the fact that populations are always finite in number. The corollary is that there is a limit to the number of haplotypes that can possibly accumulate. There must always be fewer haplotypes in a population than the number of individuals in the population. In addition, many individuals will die before reproducing and many neutral mutations will be lost by chance alone (genetic drift). It is therefore not obvious that heterozygosity will increase uniformly until saturation.

We hypothesize that the omission of this consideration in previous work may be partly responsible for the failure of previous calculations to predict the heterozygosity that is actually observed in empirical studies and in natural populations. For example, some studies find that the number of neutral haplotypes in natural populations is "orders of magnitude" less than theory predicts (Avise 2000).

We created a simple computational model to illustrate the dynamics of neutral mutation heterozygosity. Felsenstein (1971) derives rates for the loss of haplotypes in a mutation-free model, but we are unaware of analytical investigation of this question in models with both mutation and a finite population size.

### *Our Simulation of the Species Problem*

Our method was to simulate populations of genomes of length 750 base pairs (bp) that were reproduced asexually. This approach simplified calculations and still represented some inheritance mechanisms in nature, because mitochondrial DNA (mtDNA), for example, is transferred through the maternal line in mammals, without sexual recombination (Avise 2000). In canids, mtDNA analysis is widely used to support inferences about speciation. (Source code for this simulation is available from http://hampshire.edu/lspector/whatwolf.)

During reproduction, the probability of random base substitution (mutation rate) was of 1 in 50,000 or 1 in 100,000 bp. The genome length was chosen to be similar to lengths of regions commonly used for molecular dating, for example the 672 bp hyper-variable region of the mtDNA D-loop in dogs and wolves (Tsuda et al. 1997). The

mutation rates were chosen to be of the same order of magnitude as empirically determined rates, for example the rate of 1 in 85,190 bp determined by Heyer et al. (2001; they observed 0.0079 substitutions per generation per 673 bp), or the rate of 1 in 20,130 bp determined by Parsons et al. (1997; they observed 0.0303 substitutions per generation per 610 bp).

For each simulation, we began with a fixed number of clones of a randomly generated individual founder of the population. We then generated the same fixed total number of offspring in each subsequent generation, with the single parent of each offspring chosen randomly from the members of the previous generation. This produced a Poisson distribution of litter sizes with a mean of one. We continued this process for 5,000 generations while tracking the number of distinct haplotypes and the number of genomes identical to the founder.

We conducted a total of 64 simulations. Two simulations were run using different random number generator seeds for each combination of mutation rates (above) and the following population sizes: from 5,000 to 20,000 (in increments of 5,000) and from 50,000 to 600,000 (in increments of 50,000). (Note that the present-day population of wolves, worldwide, is probably between 200,000 and 300,000; see Table of wolf populations in 2000 [Boitani 2003]).

These were computationally intensive simulations, six of which terminated early due to system problems and were not re-run; we report on the results of the 58 simulations that terminated normally.

The first result was that the founder haplotype was lost early in our simulations of reproductions. The founder's haplotype was extinct as early as generation 365 and never survived to generation 2,000 (Fig. 1.2.2). The founder had the highest probability of lasting for the most generations since the first several generations consisted almost entirely of its clones; haplotypes that arose later from mutations generally became extinct more quickly, and they rarely lasted 1,000 generations.
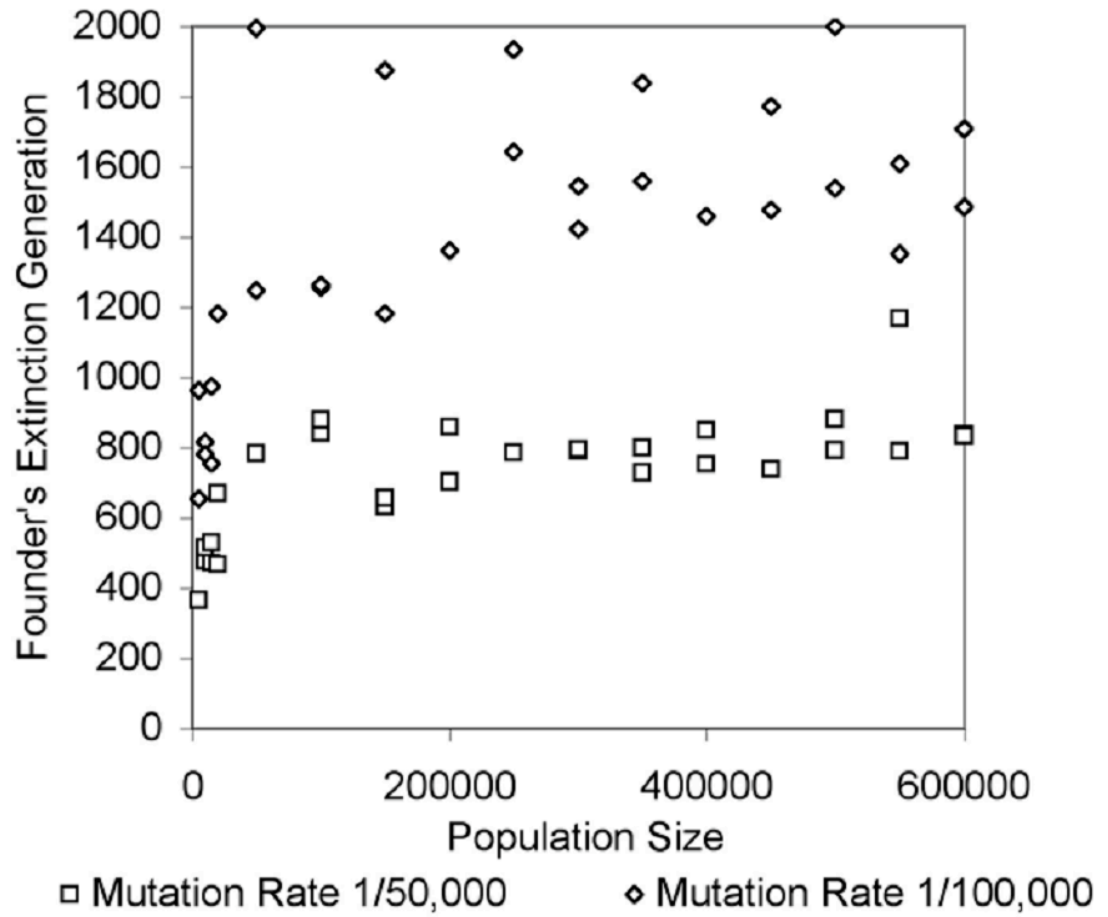
Figure 1.2.2. Extinction generations of initial founders in simulations of neutral mutation with fixed, finite population sizes.

The second result of our simulations was that the increase in number of haplotypes (a proxy for heterozygosity) attenuated in early generations. The generation in which the number of haplotypes stabilized depended on the mutation rate and on the population size. We calculated the "stabilization time" to be the first generation in which the number of haplotypes in the population was within one standard deviation of the mean number of haplotypes over the last 1,000 generations of the simulation. Figure 1.2.3 shows that in all cases stabilization occurred long before the window over which the final mean of number of haplotypes in the population was calculated (generations 4001-5000). Although stabilization occurred later when populations were larger, this effect decreased as the population grew. Simulations with much larger population sizes would stabilize in numbers of generations not much larger than those observed here. The populations in our simulations had no geographic structure; that is, the parent for each offspring was chosen randomly, without regard to geographic location. If geographic structure were added then the "effective population size" (Avise 2000) would be lower and stabilization would be correspondingly more rapid.

The third result of our analysis was that the mean number of haplotypes, averaged over the final 1,000 generations, rose linearly with the fixed, finite population size of that particular simulation (Fig. 1.2.4). In fact, after stabilization the number of unique haplotypes in a population was a function of the population size, but not of the number of generations.

One unrealistic aspect of our primary simulations was that our population sizes were held constant throughout each run, whereas natural populations are subject to niche size fluctuations and periodic population crashes due to food scarcity, disease or other cataclysmic events. To determine the effect of such events we conducted additional runs in which the population size was varied from generation to generation. We started with populations of 50,000 and each generation
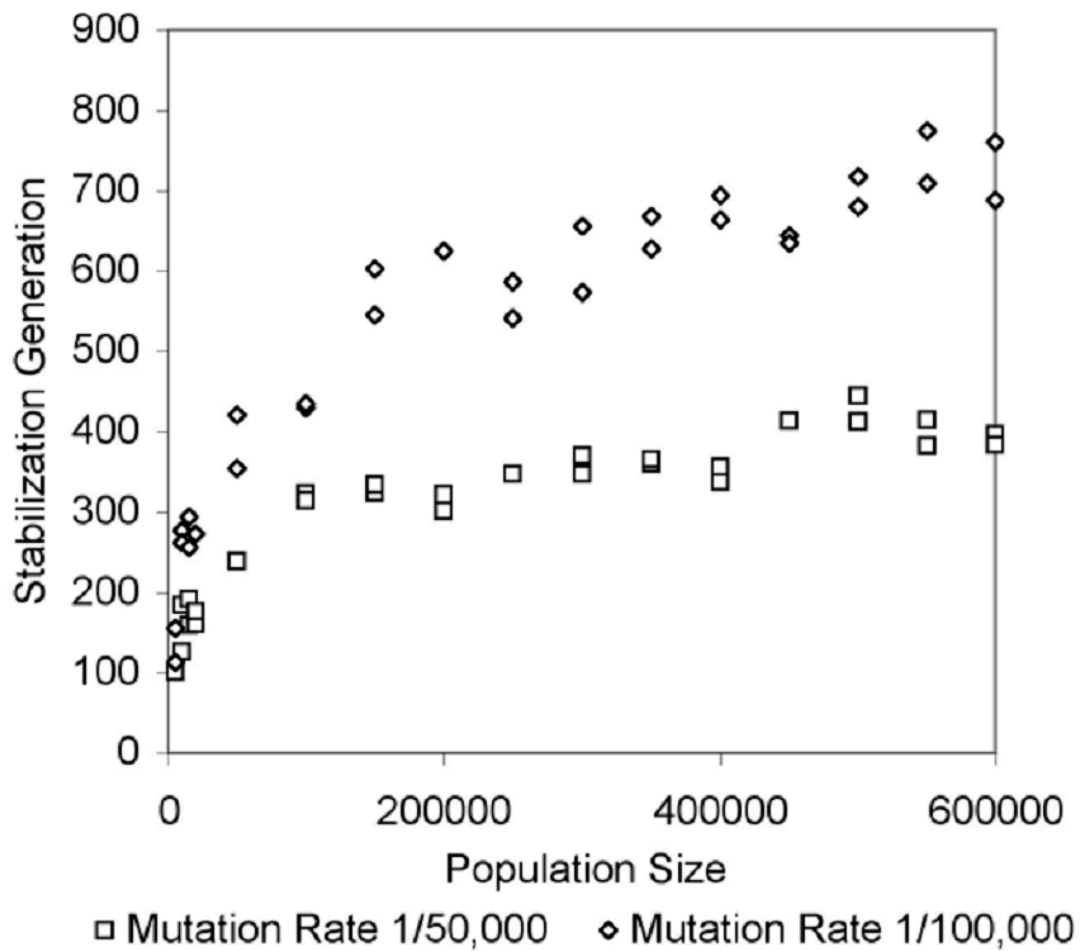
Figure 1.2.3. Stabilization generations of number of haplotypes in the population calculated with simulations of neutral mutation with fixed, finite population sizes.

changed the population size by a number chosen from a uniform distribution between -1,000 and 1,000, maintaining a minimum population size of 100 and a maximum of 100,000. A plot of population size versus number of haplotypes for all runs was created by sampling the data at 100-generation intervals from generation 2,000 to 3,000 (Fig. 1.2.5). The linear relationship was maintained even with population size fluctuation; the coefficients of determination for linear regression ($r^2$) were >0.9982 (p < 0.001) for the runs with mutation rate 1/50,000, and >0.9918 (p < 0.001) for the runs with mutation rate 1/100,000.

In analyzing the results listed above, we concluded that they have negative implications for the use of mtDNA to infer cladistic relationships, places of origin, and times of divergence. For example, Aggarwal et al. (2003) argue that Indian wolves are of ancient origin, suggesting 1-2 million years ago (ya). Vilà et al. (1997) contend that dogs diverged from wolves 135,000 ya. Wilson et al. (2000) hypothesize that *"DNA profiles of eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf,"* and over a million years ago. How is it that these papers can suggest such ancient origins when theory would predict that all haplotype information is lost within a thousand generations (in the case of wolves, 2-3,000 years)?

In another classical example of canid research, Savolainen et al. (2002) claim that since the greatest diversity of mtDNA haplotypes of dogs now exists in East Asia, therefore this must be the area of origin. Similar considerations are employed in human research when Ingman et al. (2000) reason that the higher diversity of mtDNA haplotypes in African populations *"provides compelling evidence of a human mtDNA origin in Africa."* However, Templeton (1999) has criticized such arguments with the observation that mtDNA diversity within Africa is higher in food-producing groups than in hunter-gatherers. He argues (we think correctly) that differences in genetic diversity in humans
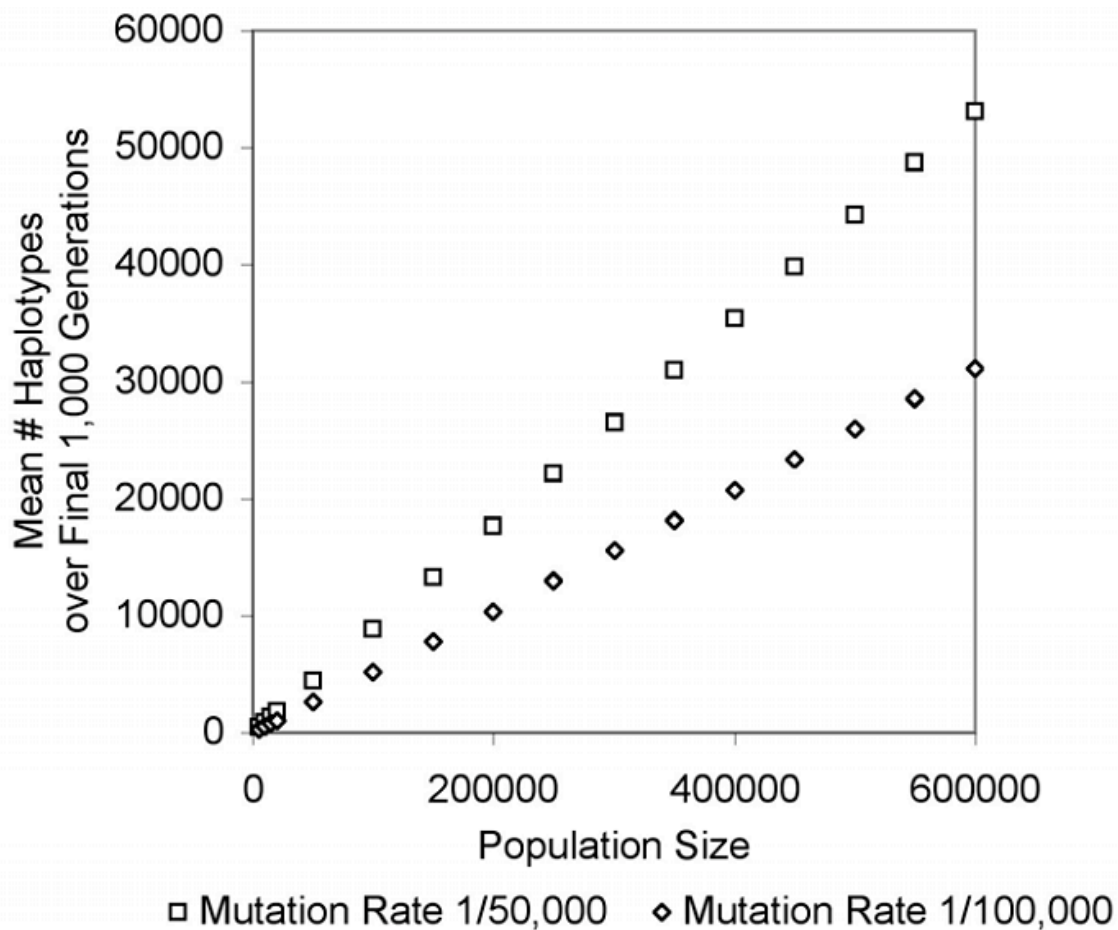
Figure 1.2.4. Mean number of haplotypes in the population over the final 1,000 generations (generations 4001-5000) calculated with simulations of neutral mutation with fixed, finite population sizes.
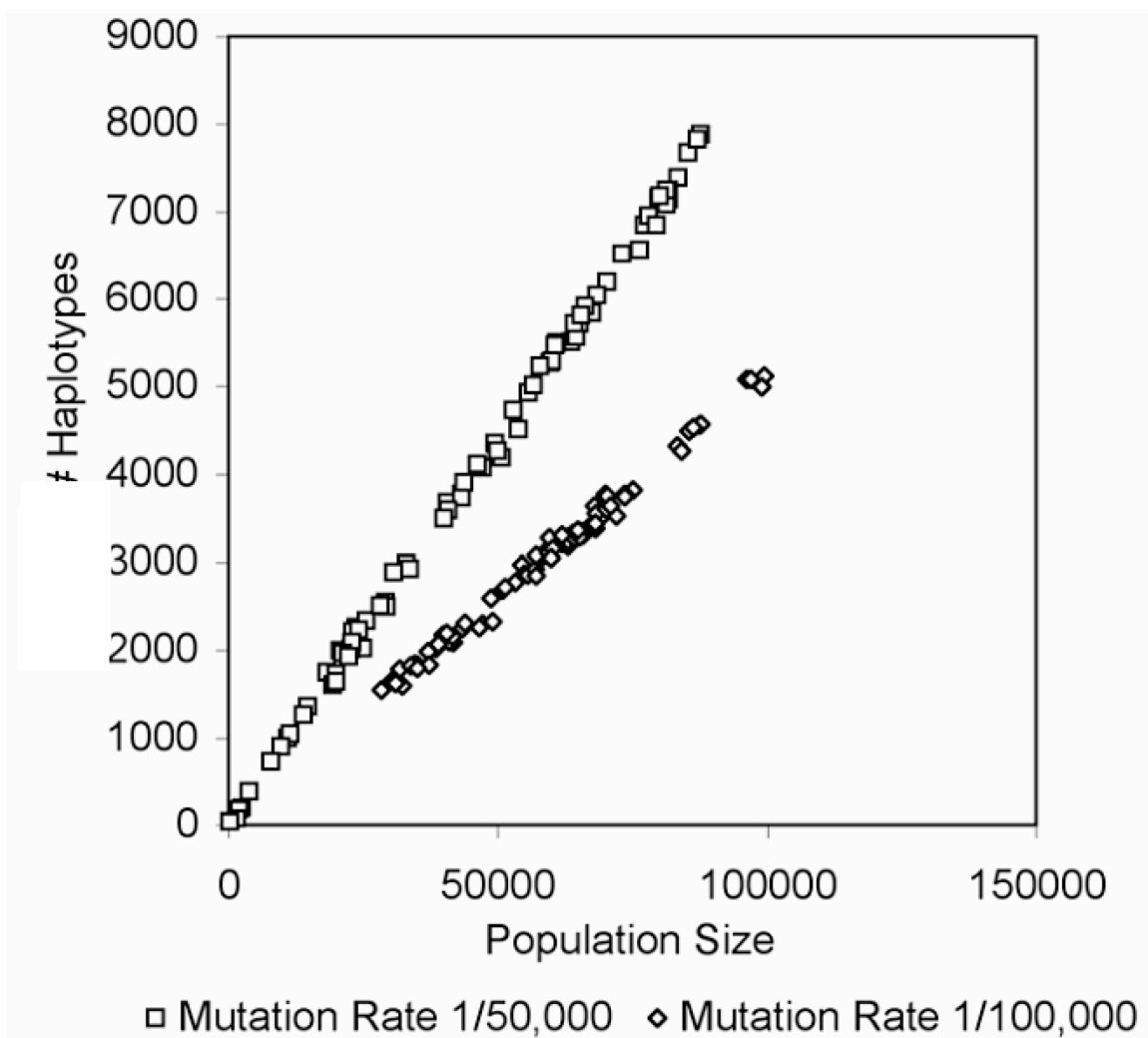
Figure 1.2.5. Number of haplotypes in the population sampled at 100-generation intervals from generation 2,000 to 3,000 during simulations of neutral mutation with variable population sizes.

are likely to be due to differences in population size and growth rates. More generally, these results may help to explain the reason for some long-debated discrepancies between theoretically predicted and empirically observed levels of heterozygosity (Lewontin 1974; Avise 1994).

In our opinion, the neutral mutation theory is seriously flawed in determining species, phylogenetic relationships, time of divergence, or the place of origin in canids, at least when mtDNA is used to obtain such inference. The best one can glean from these studies is that all the members of the genus *Canis* share haplotypes, indicating that they are simply subspecies of one another. Many biologists, including Darwin, tried to warn us of a commitment to the designation of species (see opening quote). The caveat may still exist. For example, Wilson et al. (2000) and Kyle et al. (2006) wrestle with both a genetic and a morphological solution to the question of species of eastern North American wolves. What the data showed could be that the eastern wolves were not a sexually isolated population, nor were any of the populations that surrounded it: grey wolves, coyotes or red wolves. Indeed the data could illustrate that these are subspecies or races within the genus.

### *Subspecies, Adaptation, Clines*

Mallet (2001) traces the history of taxonomy from Linnaeus to the present, discussing the different motivations for taxonomists to use a trinomial nomenclature (e.g. dog: *Canis lupus* subsp. *familiaris*). A subspecies is a non-random distribution of alleles, geographically based. A subspecies also is a variety (breed, race) within the sexually isolated species, but not in itself sexually isolated from other subspecies.

It is assumed that the observable phenotypic variation in morphology, defined by the allelic distribution, is the result of local adaptations. The Darwinian belief is that varieties, races, or subspecies are incipient species and are on the path to becoming true and new species. However, such an assumption has not a guaranteed outcome. Richard Goldschmidt (1933, as cited in Gottlieb 1992) selected for allelic differences for twenty-five years, trying to create a new species, and was never able to produce anything except subspecies, varieties, or races of gypsy moths.

This is not to say that on large continents, subspecies could not become species after separation for thousands of years, or that in some sense sympatric speciation could not happen (see Berlocher & Feder 2002), but rather that it has not been clearly demonstrated. To us, the emergence of dogs surrounded by the ancestral population of wolves might be a good example, but dogs are technically not a new species, and they are not reproductively isolated from the rest of the genus. But, whether considered species or subspecies they do demonstrate extreme divergence from the genus, obviously derived sympatrically.

There is a tendency for a genus (or a species) such as *Canis* to grade morphologically from one end of its range to another. Many mammalian species tend to grade from large sizes in the northern, polar regions to smaller size in equatorial latitudes. Single genus clines are typical of a number of North American taxa, e.g., the cervids, ovids, bovids, and of course the canids. Each of the locally-adapted populations (ecomorphs) of the *Canis* cline has been subdivided into numerous species, subspecies, and geographical races (Brewster & Fritts 1995) which are dwarfed by the sheer numbers of domestic dogs. It is often easier for ecologists to deal with the various ecomorphic

forms as if they were species—even if they are not. For example, it would be difficult for us to think of the red wolf, which is often considered as a species, as having been geographically isolated from all other 'species' of *Canis* for some Darwinian time span, and having become a sexually isolated population as a result.

Although the larger members of the genus *Canis* exist in the northern or cooler latitudes, small populations of >20 kg animals can exist as historical remnants of past climatic eras. These relic populations are not different species than the <20 kg morphs that now surround them. There is no need to think of size or coat colour as species specific, or even as adaptive, without other evidence. Such characteristics may have been adapted to an earlier climatic regime and/or simply the result of local founder effects (Geist 1992).

Wolves, meaning populations of *Canis* which are >20 kg, are going extinct in Georgia, Alabama and northward, and are being replaced by a healthy population of <20 kg *Canis*. The few remaining red wolves are not a different species than the coyotes replacing them, and indeed they breed with them, leaving their genes in a smaller ecomorph. Whatever environmental niche the red wolf morph was adapted to 5,000 years ago continues to change locally. Restoring that morph to its niche (which may no longer exist) might not be possible.

### *Our Simulation of Founder Effects in the Subspecies Problem*

Founder effects in geographically dispersed populations can be illustrated with simulations that depend upon various parameters, which were arbitrarily selected here. We modelled animals as marbles that roll around on a tabletop that represents a species niche. The tabletop is initially empty, corresponding to a niche that has recently been opened for colonization, for example by a receding glacier, or some cataclysm that locally wiped out all the previous occupants. (Source code for this simulation is available from    http://hampshire.edu/lspector/whatwolf.)

Each marble is characterized by three genes, each of which has four alleles. For the sake of visualization we map the values of the genes to colour components of the marbles, with the three genes determining the amounts of red, green, and blue. So for example if the alleles are labelled [0,1,2,3] and if these map to the amounts of colour [none, a little, a lot, full], then a marble with the genotype "000" would be coloured with no red, no green, and no blue (that is, it would be black), whereas a marble with genotype "102" would be coloured with a little red, no green, and a lot of blue (i.e. it would be dark purple).

Neither the genes nor the colours affect fitness, and both are neutral with respect to selection (neutral mutation theory). Our marbles are not inert, however. The simulation proceeds in small units of time called "time steps" and at each time step, each marble exerts a small random force in a random direction, so that the marbles tend to wander around the niche. Each marble also has a small probability (1/150 in the simulations reported here) of producing offspring. The offspring appears above the parent and usually hits the parent as it falls to the table, with the result that the parent and the offspring roll away from one another.

We explored two conditions of inheritance. In the first condition the offspring's genes are copied from the single parent's genes and each gene is then mutated with a probability of 1/100. Mutation is performed by changing a gene to a randomly chosen

allele, which has a 1/4 probability of being the same as the original allele. This corresponds either to asexual reproduction or to single-sex propagation of genetic material, as occurs with mitochondrial DNA. In the second condition we permitted hybridization: a "mate" is chosen randomly from the nearby marbles and each gene is taken (with possible mutation) either from the parent or from the mate, each with a probability of 1/2.

A marble "dies" and is removed from the system if it reaches the age of 250 time steps, and it "ages" an additional 10 time steps each time it collides with another marble. This aging process helps to ensure that the population will spread across the table, since tightly grouped marbles will die more quickly and will therefore produce less offspring. This feature can be considered a model of a simple local resource, open space.

We begin each simulation with a single marble and we observe the dynamics of the system as offspring are produced and the population grows. If all of the marbles die out, which is rare with the parameters that we used except near the start of a simulation, then we start over. We limit the population to 1,000, but in the simulations reported here the populations grew only to about 800 and the limit was never reached.

Early in each simulation one observes a small and nearly homogeneous population with a few individuals having different genotypes that were produced by mutation (Fig. 1.2.6). After a few thousand time steps the population will have spread across the tabletop and several geographically distinct and genetically unique subspecies will be evident (Fig. 1.2.7).

Ecologists have developed a variety of tools with which to measure the emergence of geographically distinct subspecies (for example see Hubbell 1997). Here we use a simple measure of "localization" calculated as follows. We divide the tabletop into a 10 x 10 grid, and for each genotype we count the number of grid squares in which marbles with that genotype do not occur; this number is called the "vacancies" of the genotype. We then sum, across all genotypes, the number of marbles with each genotype times the vacancies of that genotype. This summation is then divided by the total population size and then again by 99 (the number of grid squares minus one) to produce an overall
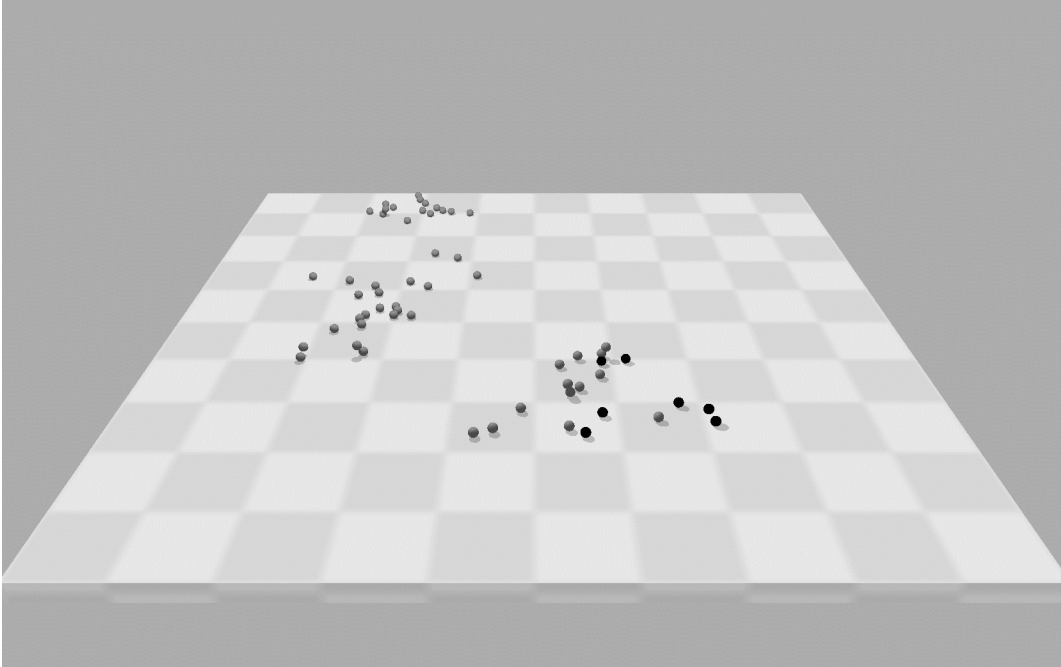
Figure 1.2.6. Early snapshot of a "virtual marble" simulation demonstrating founder effects, with colours reduced to shades of gray. A small and nearly homogeneous population can be observed with a few individuals having different genotypes that were produced by mutation.
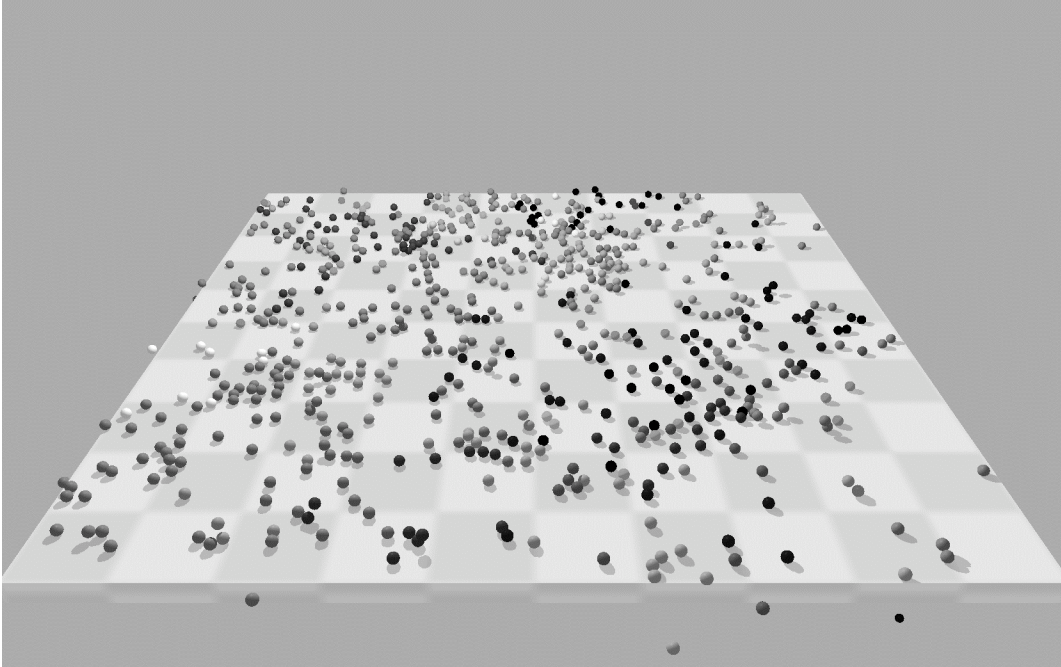
Figure 1.2.7. Later snapshot of a "virtual marble" simulation demonstrating founder effects, with colours reduced to shades of gray. A comparison with Fig. 1.2.6 shows that the population has spread, and subspecies can be identified as geographically and genetically distinct groups of individuals.

localization coefficient that ranges from 0 to 1. A localization coefficient of 0 means that all genotypes occur everywhere across the table top, while a localization coefficient of 1 means that each genotype occurs only in one grid square. In general, the localization coefficient can be thought of as the percentage of the territory in which a typical marble's genotype will not be found.

       We plotted the localization coefficient values of ten simulations (five with hybridization, averaged together, and five without hybridization, averaged together) each of which was run for 10,000 time steps (Fig. 1.2.8). Localization was always initially 1, as the initial founder was perfectly localized. It dropped as the population grew and spread, but it rebounded. At the end of each simulation the approximately 700 marbles were segregated geographically. We also plotted the numbers of subspecies in the same simulations (Fig. 1.2.9).  Our primary observation from running these simulations was that geographically distinct subspecies readily emerged, even without adaptive selection of any sort. This has also been shown in human populations (Novembre et al. 2008). Also note that the hybridization condition produced more subspecies in our simulation.
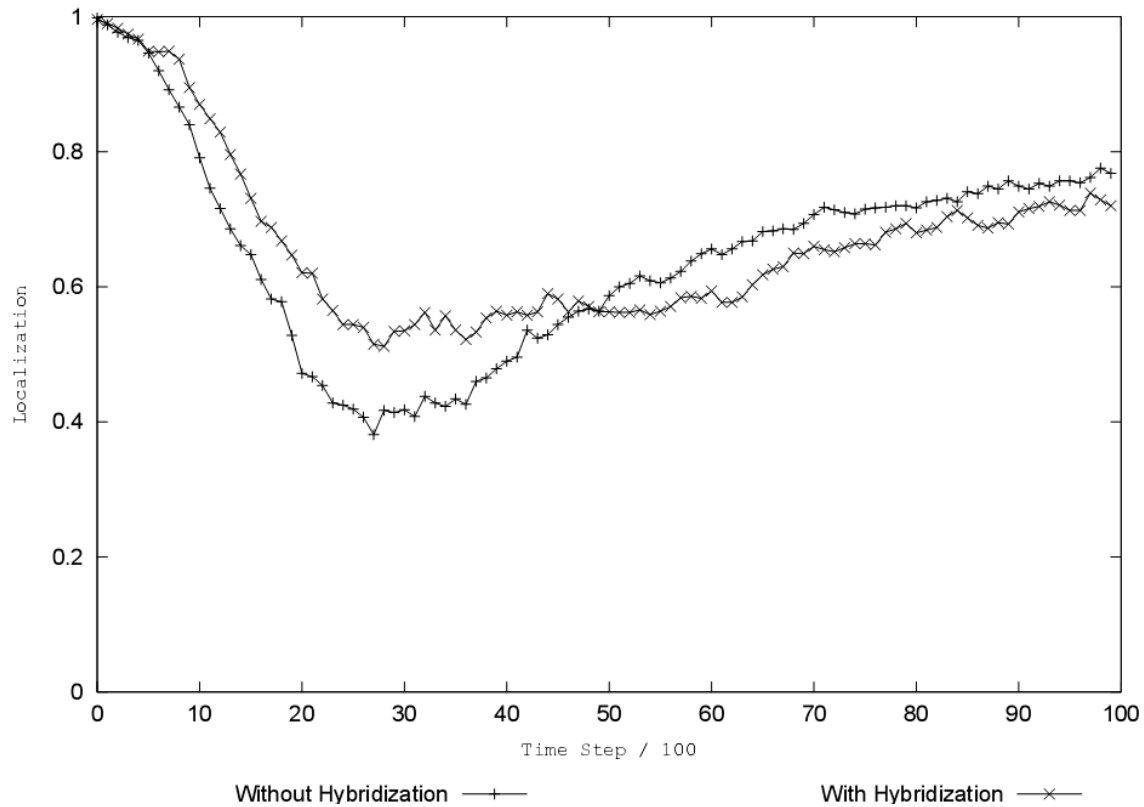
Figure 1.2.8. Localization coefficient values for ten "virtual marble" simulations run for 10,000 time steps (five with hybridization, averaged together, and five without hybridization, averaged together). Localization was initially 1, as the initial founder was geographically localized. At the end of simulations, the marbles, representing individuals, were again localized demonstrating founder effects.
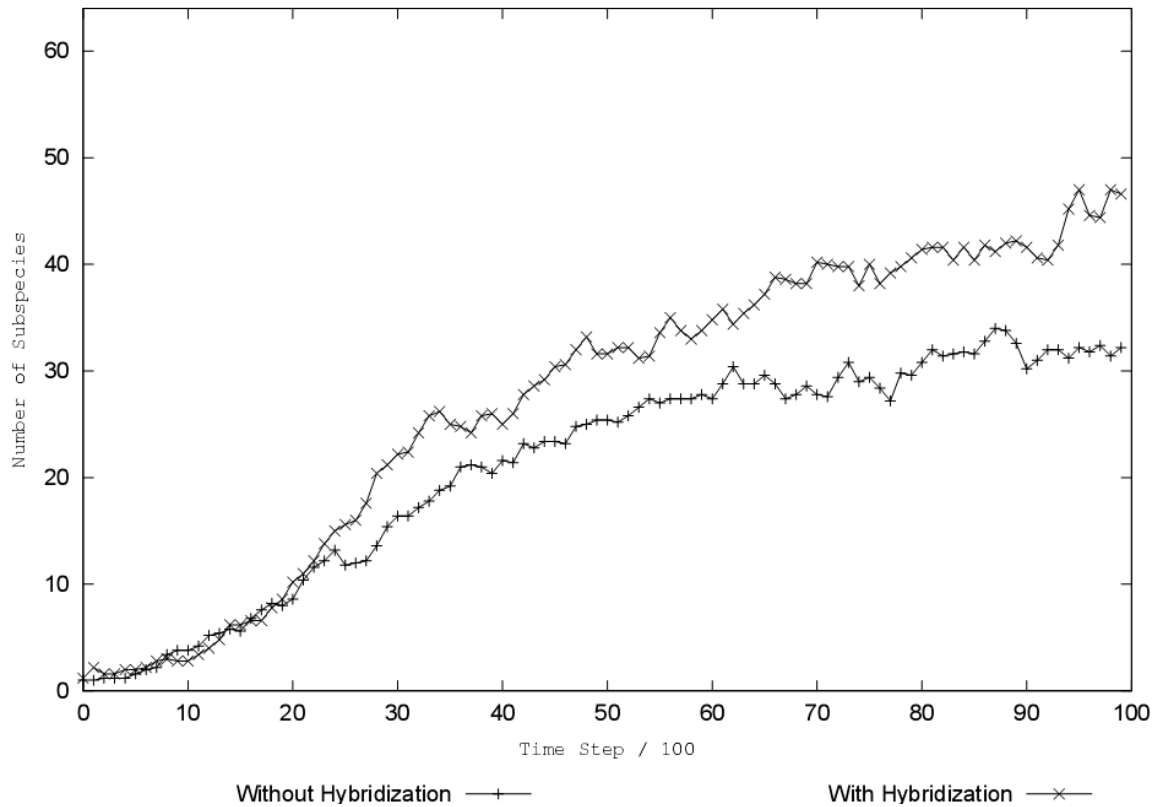
Figure 1.2.9. Numbers of subspecies obtained for ten "virtual marble" simulations run for 10,000 time steps (five with hybridization, averaged together, and five without hybridization, averaged together). Results of Figure 1.2.8 and this figure indicate how geographically distinct subspecies emerge, even without adaptive selection. The hybridization condition produced more subspecies.

The model illustrates why there can be so many subspecies represented in any population of animals with worldwide distributions such as those of the genus *Canis*. It also illustrates that the gene frequency at any given location is continuously changing in response to population shifts. If the local population does not go to zero, vacancies will be repopulated by individuals that do not represent the entire genetic spectrum of the individuals that are being replaced (founder effects).

### *Hybridization in Nature and Phylogenetic Webs*

*"...[there is] every reason to believe that new species may arise quite suddenly, sometimes by hybridization, sometimes perhaps by other means. Such species do not arise as Darwin thought, by natural selection."*
Haldane 1956

*"...most speciation involves natural selection; natural selection requires genetic variation; genetic variation is enhanced by hybridization; and hybridization and introgression between species is a regular occurrence..."*
Mallet 2007

Just as the term species gets misused, it is much the same with hybridization. In the classic biological literature, species hybridization is characterized by a karyotypic change. The offspring or the new species is a polyploid or an alloploid of the parent species (Mallet 2007). In the *Canis* literature we tend to use the term hybridization in the agricultural sense of the word, where it is the crossing of races or breeds. In that sense the product is a mongrel but not a new form in the species sense because it never leads to sexual isolation the way karyotypic change does. In the *Canis* literature the term hybrid gets used in the agricultural sense but with the consequences of the biological usage.

For those of us involved in *Canis* conservation, each time two of the presently described "species" hybridize, the fear is that we will lose the "species" with the smallest population. But what is being lost is the phenotype. The genes of the individual are being passed on. The smaller population could become unrecognizable phenotypically, but that does not mean they have genetically disappeared.

Coyotes breeding with gray wolves (Mech 1970), and coyotes breeding with red wolves (Nowak & Paradiso 1983) are cases where the authors are concerned that continued hybridization will lead to the demise of the species they are trying to protect and/or restore. Boitani et al. (1995) argue that hybridization between the 200-500 wolves in Italy and the 800,000 stray dogs may be a threat to that recovering wolf population, because of not only genetic contamination, but also competition for resources. Wayne and Koepfli (1996) report that 15% of Simien wolf/jackals contain evidence of hybridization with domestic dogs. Wilson et al. (2000) report that three red wolves, one Algonquin wolf, and four southern Ontario wolves have the same mtDNA haplotype as Texas coyotes. Twenty-five percent of the animals they are trying to differentiate have coyote haplotypes. For Vilà et al. (1997), nearly 20% of their dog breeds have wolf mtDNA. Lehman et al. (1991) found wolves with coyote mtDNA, and Wayne and Jenks (1991) identified "all" their red wolf population as containing coyote and/or grey wolf mtDNA.

Each genetic study of a *Canis* species seems to have to deal with the hybrid problem. In each study there is the *a priori* assumption that their animal is a true species and qualifies for the binomial nomenclature given to them. In each case it is assumed that sharing haplotypes is evidence of hybridization of the "species." However, we propose two different conclusions: 1) these are not true species, but rather subspecies of one another, and/or 2) the various methodologies cannot discriminate between them. As subspecies the expectation is that they will have a gene flow between them. In the true agricultural sense of the word hybrid there will be interbreeding between the various races, breeds, varieties, and subspecies of the species. And because they are hybridizing, the ability of neutral mutation theory to discriminate between them is impossible. In addition, as in our diagram on skull shapes (Fig. 1.2.1), cladistic mitochondrial relationships may not be evidence of phylogenetic relationships, but rather of local founder effects.

Lorenzini and Fico (1995) cited several works of Boitani and his colleagues in Italy, who *"consider the interbreeding with domestic dogs one of the major threats to the integrity of the gene pool of the Italian wolf."* At a canid conference, one reporter outlined a project that was designed to keep red wolves pure (Adams et al. 2001). The founding red wolves in the recovery program were genotyped using microsatellite loci, so that wildlife managers will be able to sample the recovering population and weed out any red wolf offspring that show coyote or dog mtDNA. A similar approach has been proposed for the Ethiopian highlands, to neuter local dogs and reduce the flow of genes to the Ethiopian wolves (Laurenson et al. 1997).

However, there is a growing literature that suggests that hybridization might be a major source of genetic variation in nature (Reyer 2008). In fact, right from the beginning of Darwin's theory of transmutation of species by natural selection, critics argued that natural selection would decrease the variation from which further selection would proceed (Mivart 1871). The argument that natural selection decreases phenotypic variability—Darwin's theory is based on phenotypic variability—persisted until the twentieth century discovery of genes and then gene mutation. The increase in genetic variability comes from two sources—recombination and chance mutation. Hybridization maximizes recombination.

Lewontin and Birch (1966) suggest that hybridization is a major source of variation for adaptation to new environments. (New environments can be changing environments.) Haldane (1956) goes further in suggesting that hybridization can be a source of new species. Mallet (2007) concludes that *"hybridization can contribute to adaptive radiations..."*. (An adaptive radiation is a rapid evolutionary process characterized by an increase in the morphological and ecological diversity of a single, diversifying lineage.) Coppinger and Coppinger (2001) suggest that hybridization is a way to create forms that are phylogenetically bizarre. Almost all of our modern breeds of dogs in their most divergent forms are creations of sub-specific hybridization. Arnold (1997) summarizes a large literature on natural hybridization, giving many examples from the literature on plant, insect, lizard, mammal (coyote, wolf) and bird hybridization. Most important, he attacks the philosophical notion originated by Darwin and promoted by Mayr (1982) and others that hybrids must always be irrelevant to evolution. In fact, Arons and Shoemaker (1992) demonstrate the idea of novel phenotype as a product of hybridization.

In any restoration program, the success or failure is partly the ability of the restored animals to adapt to the habitat. The population being restored is small or rare, meaning a small gene pool and founder effects. Hybridization may increase the individual's fitness and create novel behaviour patterns, which allow for novel adaptation to new habitats (Arnold 1997; Doolittle 2005). Since hybridizing processes are occurring naturally in the genus *Canis,* it could prove counter-productive to their genetic survival to try to further isolate them from diversifying through hybridization with peripheral (and successful) forms (Kyle et al. 2006).

Once one realizes that existing "species" can swap genetic material and use that material in adaptive ways, the idea of a phylogenetic tree may become obsolete (Doolittle 2005). For those of us trying to understand the evolution of the various forms within the genus *Canis*, it all of a sudden becomes clear why constructing cladograms and phylogenetic trees is so difficult. These organisms are sharing genetic material and probably have for the last 5,000,000 years. Within the genus very little radiation has taken place. They change size constantly, but essentially the phenotype is very conservative (Radinsky 1981). In fact, the various forms of *Canis* may not be monophyletic species as is indicated in our Fig. 1.2.10.
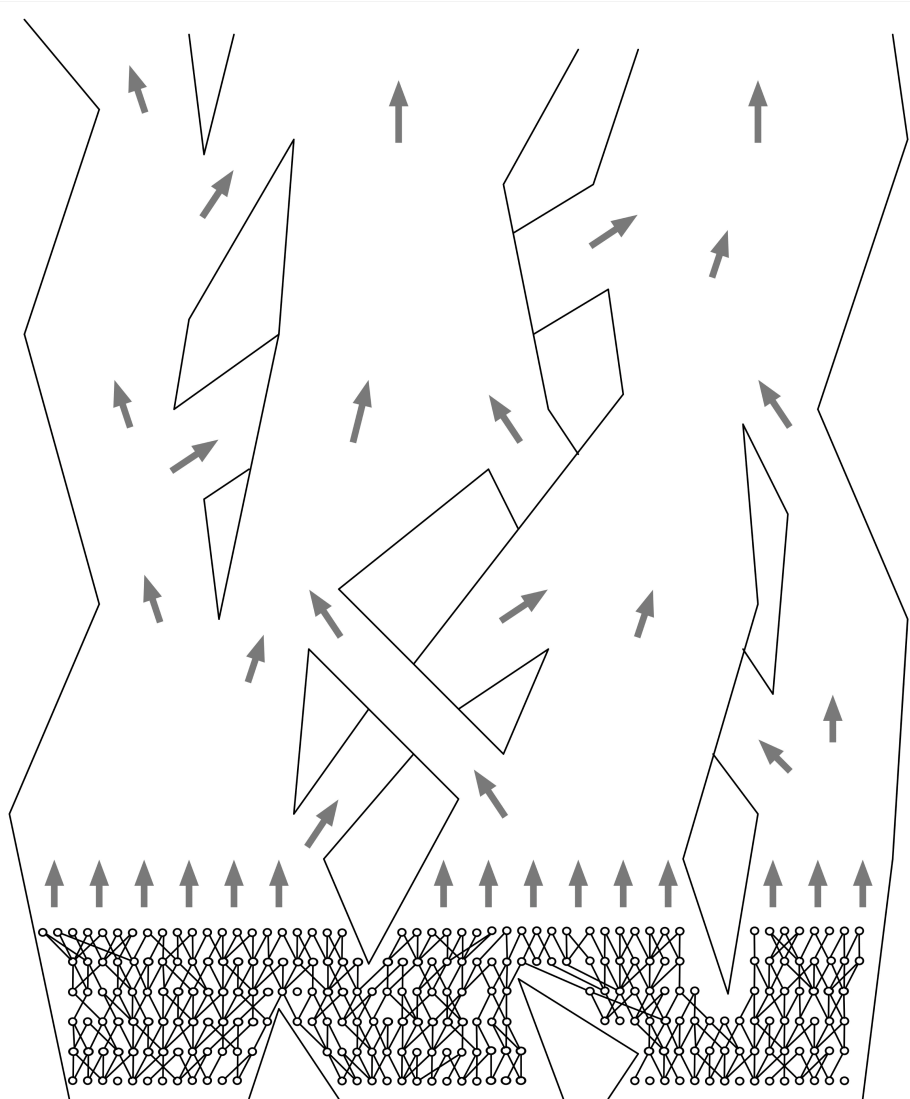
Figure 1.2.10. Schematic, conceptual diagram illustrating a potential phylogeny of a taxon, the genus *Canis* for example. Lines represent apparent boundaries between species; lines connecting circles (individuals) represent family trees from one generation to the next (time progresses upward); arrows represent family trees diverging into separate species or converging into lumping species.

## *Conservation Management*

The genus *Canis* presently is divided into seven or eight species. All were named before Darwin's theory of evolution. The now-designated species do not come up to the criteria of species designation for sexually isolated populations. The members of the genus *Canis* are karyotypically identical and they interbreed, producing viable offspring in the wild. Morphometric measurements indicate phenotypic differences, but those differences rarely are indicators of species differences. Genetic studies have shown a gene flow between existing populations and species.

From the point of view of conservation management, *Canis* could be recognized as subspecies. Restoration programs could encourage viable populations of the genus *Canis* in the habitats they wish to restore. From a practical point of view, if there is still the wish to call these populations wolves, or red wolves, or Algonquin wolves (see above), for popular or political reasons, then so be it. Subspecies may be protected too, as it is exemplified by the Endangered Species Act in the U.S.

Hybrids may be classified as good subspecies and should not be necessarily discriminated against in conservation programs. Experimental evidence using mtDNA and cladistic statistics models demostrate that gene flow is constant and "pure" species are wishful thinking. In *Canis*, hybridization may increase genetic variability and in some instances create phenotypic novelties. Thus, hybridization should not be artificially prevented in restoration programs. Small populations of animals need genetic diversity for adaptation to occur. They also need genetic diversity to avoid deleterious bottlenecks that are the inevitable result of the restriction of gene flow.

The five noted biologists who are quoted at the tops of sections in this chapter were unencumbered during their years of investigation by the need to determine how a species should—or must—be preserved. In fact, they understood that the binomials and trinomials existed only as convenient labels, abstract concepts, approximations of reality. The species of the genus *Canis* in the world are the results of standing before Haldane's (1932) "tribunal of natural selection." The wolf—in whatever morphological or genetic phenotypes it has achieved—has maintained its Aristotelian essence in spite of our management.

## *Acknowledgments*

## *Additional References*

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