1.2   What, if anything, is a Wolf?

Raymond Coppinger, Lee Spector and Lynn Miller

"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)

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Introduction

Species are forever a moving target. In every generation, there should be an adaptive response of a population of animals to an inconstant world. To take just one example, some work with Darwin's finches (Grant & Grant 1999) shows enhancement of genetic variation, with new phenotypes to be more fit in new habitats after the major climatic perturbation of an El Niño event.

This biological perspective, which is the core of the theory that has unified biology ever since Darwin proposed natural selection, sometimes seems to be forgotten in the face of practical management decisions. 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 population, implies that the essence (Aristotle, Linnaeus) of the species is conserved. It should never 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, the geneticists say their animal 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. In other cases, some opponents of

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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 even to professionals. Not only are species moving targets, but the definitions of species are ephemeral. The second, perhaps the 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. Attempting to provide a species or subspecies, much less a population, with legal definitions that can be used to define conservation plans that maximize the success of recovery programs, 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 this level, it is the essence of the species that is being conserved and no measurement or genetics need matter. 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. *"Although deterministic models and theories have continually been challenged and refuted, phrases such as "climax state," "stability of ecosystems" "carrying capacity," and "balance of nature" still form the core of much conservation and environmental rhetoric"* (Drury 1998).

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And at a third level, the attempt is the preservation of biodiversity, to maximize biodiversity as is elegantly laid out by Wilson (1999). Importantly in 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 some number of species now, and our job as wildlife managers is to discover, describe, and preserve as many of them as possible.

Protection and/or restoration of a species necessarily fail to consider the continuously changing characteristics of biodiversity. In theory, it is essentially impossible to "save" or restore a species as either a phenotype or genotype. The role of humans in "contemporary evolution" becomes the essential question.

We often practice wildlife management or endangered species management as if species are a fixed value. In the red wolf recovery program, animals to be released into the wild have been genetically fingerprinted with the aim of testing the resulting filial 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 lupus familiaris) (Wayne & Gottelli 1997), the proposal is to kill or sterilize the larger population in order to preserve the endangered species (Laurenson et al. 1997). 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 just vermin to be removed.

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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.

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? Why should wildlife managers believe the geneticists who claim that all red wolves are hybrids? Have we studied their methodologies and their definitions? Have they thought out the implications of what they are saying, when they say species are hybridizing? 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? The consequence of being wrong in trusting the taxonomist, the geneticist, the evolutionary theorist, is devastating when false assumptions appear in the legal language of an Endangered Species Act.

Species Identification

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

Haldane 1956

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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 one species of rodent), or that rodents and lagomorphs are descended from a common ancestor, or perhaps that rabbits were the ancestors of the rodents, or perhaps they weren't related to the rodents at all but were related to hyraxes.

Experts shift back and forth, changing their minds about ancestries—based on new evidence, and sometimes upon re-analysis of morphological measurements. In the last fifty years, molecular biology has steadily grown in technique, and enabled new insights to many evolutionary questions. In January of 1996, an article published in Nature gives evidence that rabbits are descendants of primates (Graur et al. 1996). It is the kind of discovery that could "never" have been made through skull measurements or comparison of teeth, since such phenotypic characteristics might be confounded by convergent evolution.

What difference does it make—why even bother? For most of us, it is just academic curiosity. What was the evolutionary process that led to this transmutation of some ancient primate into a rabbit? But for others, having a good definition of species and their evolutionary history is necessary for forensic and management policy. Without a proper well-defined name, wildlife law as it applies to endangered species becomes impossible.

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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).

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. However, if it is a hybrid gray wolf or hybrid red wolf, then it is not protected under the ESA and some would argue that you should shoot it so it cannot corrupt the endangered species. If it is *C. lupus lycaon*, you can shoot it in Quebec, Canada but not in the north-eastern U.S. because technically it is a gray wolf. However, if it is *C. lycaon* it is unclear what the rules are in North-eastern U.S. And if it is a coyote, you had better shoot it because if your neighbours find out you let a varmint go, you'll be ostracized. If it is a coydog, you cannot capture and keep it in Connecticut because it is a wild animal and a permit is required. In Massachusetts, anything with dog in it is a dog and you can cage it. (Since the domestic dog is technically *Canis lupus fam*., it is probably covered under the ESA.

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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?

Wolves and their relatives have a confusing taxonomic history. There are big differences between rabbits and wolves. Everyone knows what a rabbit is, even if it is not "anything but a rabbit." We might not know who the rabbit's ancestors were or exactly how they are related to their closest relatives, but we do know what one is. Wolves and their relatives present an almost opposite problem to rabbits. Every species of the genus *Canis* has similar morphological and genetic features and it is difficult to tell the differences among them. Indeed all eight (seven?) 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) Their genetic differences are allelic. All members of the genus are inter-fertile. Hybrids are easily produced in the laboratory for study. There are growing numbers of wolf/dog hybrids kept for pets. There are dog/jackal hybrids used in laboratory studies and they are trained for activities such as locating drugs or bombs.

Journal papers report that coyotes, wolves, dogs, and jackals hybridize "naturally." The only barriers to reproduction among all these species are mechanical barriers—size differences, social organizations that tend to restrict reproductive access, and of course geography.

Morphometric Solutions to the Species Problem

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"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

We should be able to measure our 20 kg animal in New England and identify it.

Traditionally, as with the rabbit/rodent problem, differentiation was based on

measurement. The underlying assumption is that statistical differences in phenotype

reflect differences in genotype. The larger the differences in phenotype, the more

distantly related are the two forms. This makes apparent sense since transmutation of

species by natural selection is a slow process. In addition, it seems to makes sense when

you are measuring basicrania, dental formulas or tooth morphology, and other

conservative organs in the body.

It is an assumption however, that phenotype represents genotype. When that

assumption is used to determine species, especially fossil species, as sexually isolated

populations, it is inadequate to the task. Dogs probably have the greatest morphological

deviations (breeds) in the mammal world, are not distantly related one to another, and are

not different species one from another. All skull growth is regulated by both allometric

ratios and accommodative effects. Very minor changes in onsets and offsets and

allometric growth ratios can result in enormous differences in the resulting head shape.

Very small allelic differences can create the brachycephalic head of the bulldog or the

dolicephalic head of the borzoi.

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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. The actual differences in distance between any two skulls were summed.

If these two charts (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 with the view.

Differences in head shape have little relationship to phylogeny (Fig. 1.2.1). One cannot infer genetic relatedness nor construct a phylogenetic tree using morphometrics. We suggest that phenotype is not good a method for measuring genotype at the species level.

Yet morphometricians continue to assume that populations of animals that have similar canonical values are more closely related than populations that are dissimilar. Morphometricians cannot distinguish founder effects nor convergent evolutionary effects. Researchers measure fossil skulls with the assumption that the canonical values are the product of genotype and the differences between ancient populations and modern forms are evidence of genetic evolution. The morphometrician assumes that the measurements are a reflection of genetics and can be used to differentiate between genetically divergent populations in order to make some judgment about speciation, cladistic relationships and
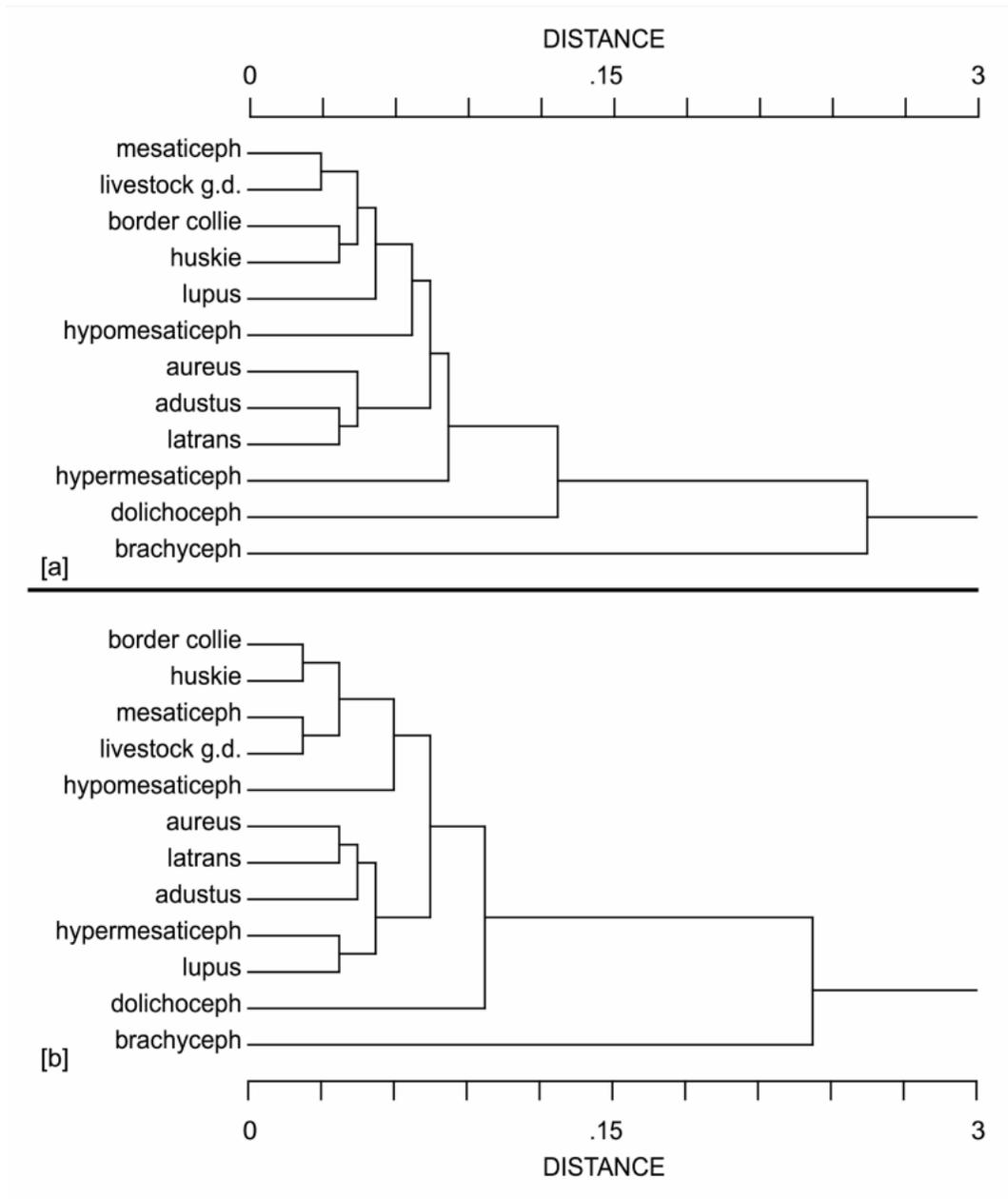
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Figure 1.2.1. Cladograms of five species of the genus Canis, including assorted breeds of dogs, based on (a) dorsal and (b) ventral morphometric views of adult skulls.

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origins. What happens when coyotes and side-striped jackals have similar canonical values? Well, that is very interesting.

Genetic Solutions to the Species Problem

In the past thirty years, molecular geneticists have entered the species debate. Their intent is to be able to find in the genes a species marker, to find sequences of genes that will show the relationships between the species, and finally to find a genetic clock that will give the dates of origins of those species. If speciation is the gradual shift of allelic frequency over time, it is assumed that populations with similar allelic structures are more closely related, while those with radically different genetic structure are more distantly related. The larger those genetic differences are, the longer the time since the two species diverged. It is a little unclear if the same reasoning could be applied to subspecies, which technically have not diverged and still have a continuous—albeit limited—gene flow within the greater population.

The geneticist argument starts with the neutral mutation theory. 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 on the extra-nuclear mitochondrial DNA (mtDNA), or within the nucleus on the Y-chromosomes, or within the micro-satellites, 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. The resulting

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increase in heterozygosity becomes an indicator or clock of phylogenetic time. One prediction is that higher heterozygosity of these neutral genes indicates an older population, i.e., a longer elapsed time since the original single haplotype, or mother of the species or group ("Eve"). Some authors also predict that centres of greatest heterozygosity are centres of species origins (Leonard et al. 2002; Savolainen et al. 2002).

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. 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. 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 rate and a finite population size.

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Our method was to simulate populations of genomes of length 750BP that were reproduced asexually with a probability of error (random base substitution, with possible replacement) of 1 in 50,000 or 1 in 100,000 base copies. The genome length was chosen to be similar to lengths of regions commonly used for molecular dating, for example the 672BP 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 base copies determined by Heyer et al. (2001; they observed 0.0079 substitutions per generation per 673BP), or the rate of 1 in 20,130 base copies determined by Parsons et al. (1997; they observed 1/33 substitutions per generation per 610BP). These rates are considerably higher than many inferred "phylogenetic" rates; we used the empirically determined rates because we were investigating the validity of the phylogenetic rate inferences.

For each simulation, we began with a randomly generated founder and generated a 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 64 simulations, two (using different random number generator seeds) for each combination of population size and mutation rate (error probability). We used 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 300,000 and

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400,000). 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. The founder's haplotype was extinct as early as generation 365 and never survived to generation 2,000. The average extinction generation of the founder, across all reported simulations, was 1,078 (Fig. 1.2.2). The founder had the best probabilistic shot at 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 much more quickly and rarely lasted 1,000 generations.

The second result was that the number of haplotypes (heterozygosity) reached a stochastic steady state 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 was within one standard deviation of the mean number of haplotypes over the last 1,000 generations of the simulation. Stabilization times ranged from 101 to 774 generations, with an average of 418 generations (Fig. 1.2.3). In all cases stabilization occurred long before the window over which the final mean was calculated (generations 4001-5000). Although stabilization occurred later when populations were larger, this effect decreased as the population grew, and we expect that 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,
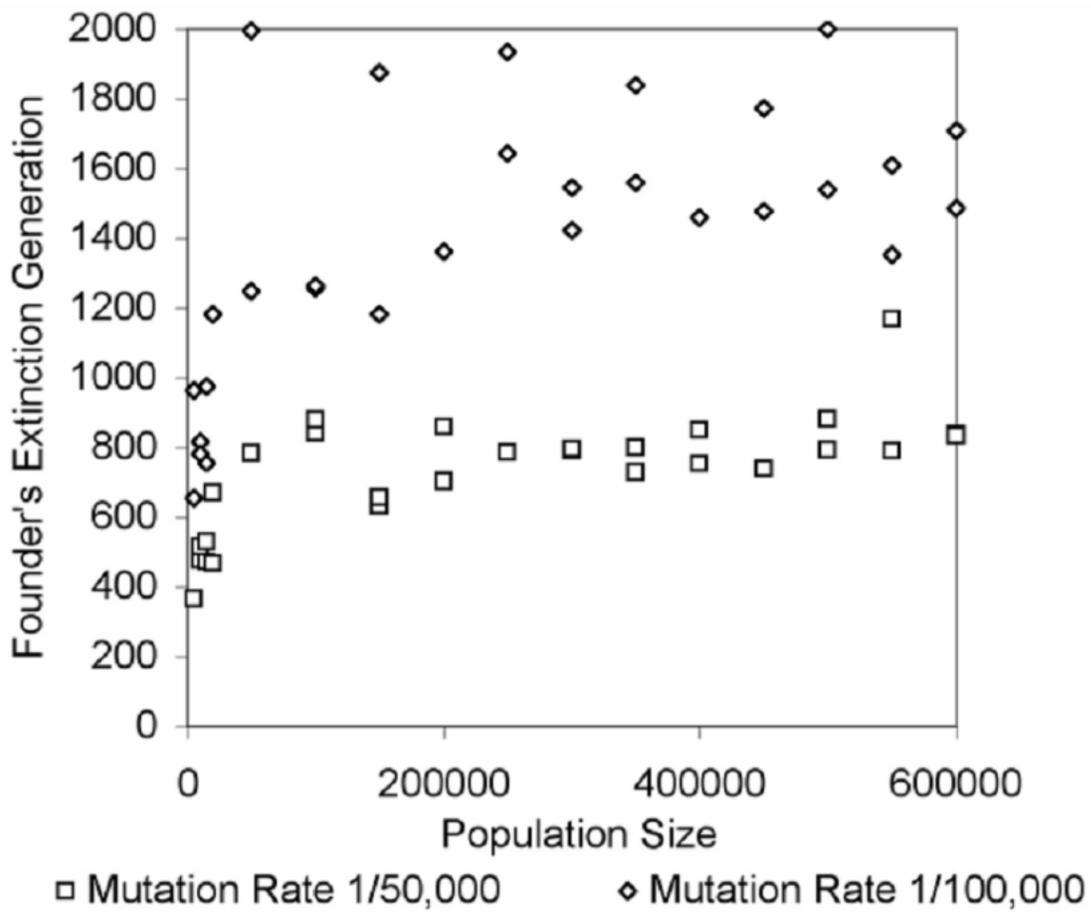
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Figure 1.2.2. Extinction generations of initial founders in simulations of neutral mutation with fixed, finite population sizes.
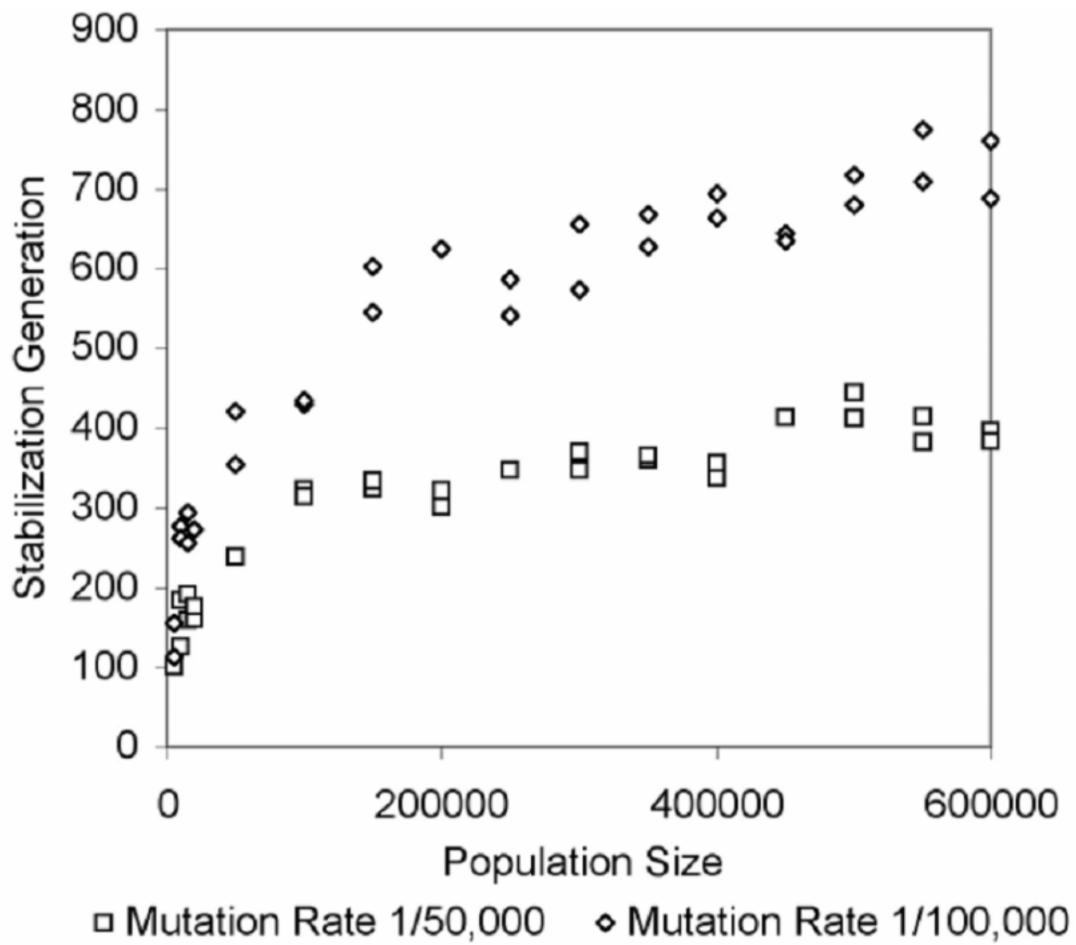
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Figure 1.2.3. Stabilization generations of number of haplotypes in simulations of neutral

mutation with fixed, finite population sizes.

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the parent for each offspring was chosen randomly, without regard to geographic

location. We conjectured that if geographic or social structure were added then the

"effective population size" (Avise 2000) would be lower and we would expect

stabilization to be correspondingly more rapid.

The third result of our simulations was that the mean number of haplotypes,

averaged over the final 1,000 generations, rose linearly with the population size (Fig.

1.2.4). 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 disease or other cataclysmic

events. To determine the effect of such events we conducted 14 additional runs (7 with

each mutation rate) in which the population size was varied from generation to

generation. We started with populations of 50,000 and each generation 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. The

stabilization metric could not be directly applied to data from these runs, but it was clear

from graphs of individual runs (not shown) that "re-stabilization" occurred rapidly and

that the number of haplotypes tracked changes in the population size. A plot of

population size versus number of haplotypes for all 14 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
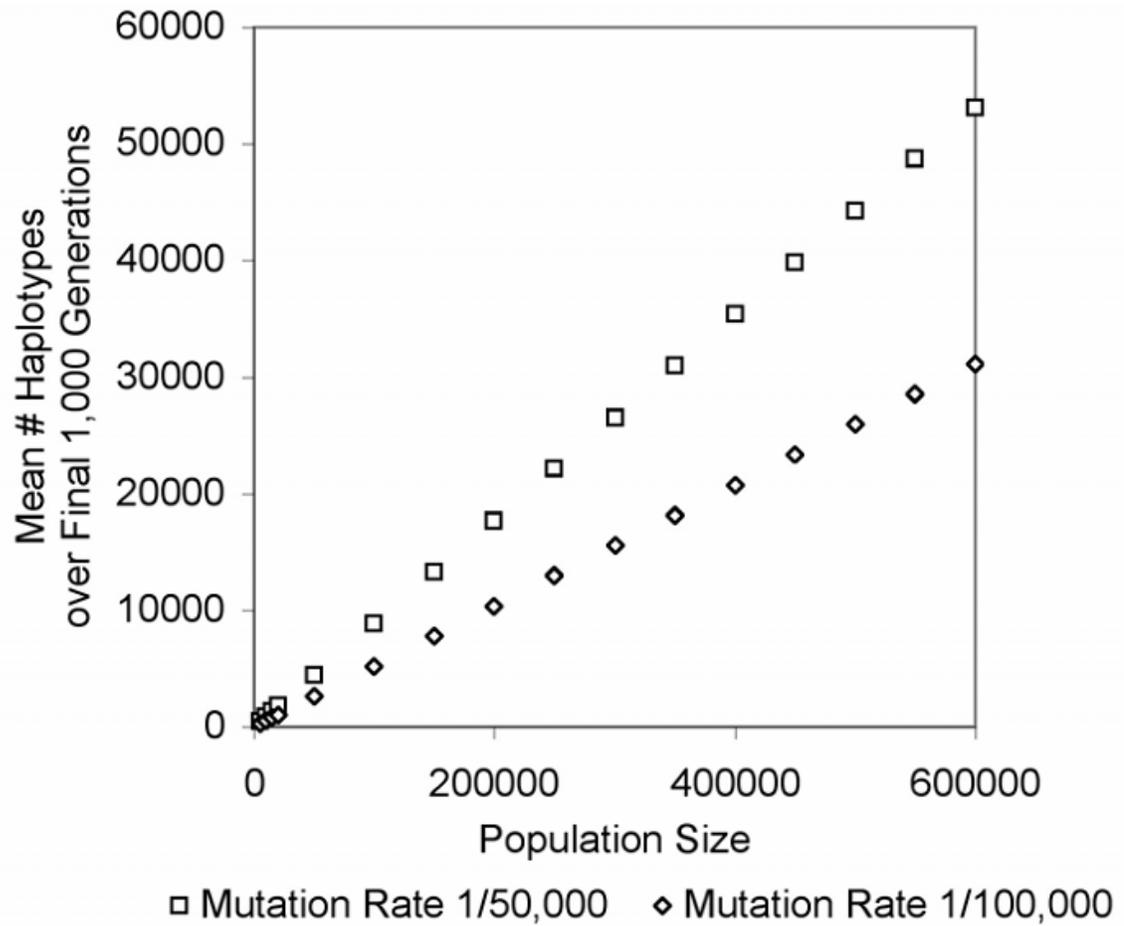
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Figure 1.2.4. Mean number of haplotypes over final 1,000 generations in simulations of neutral mutation with fixed, finite population sizes.
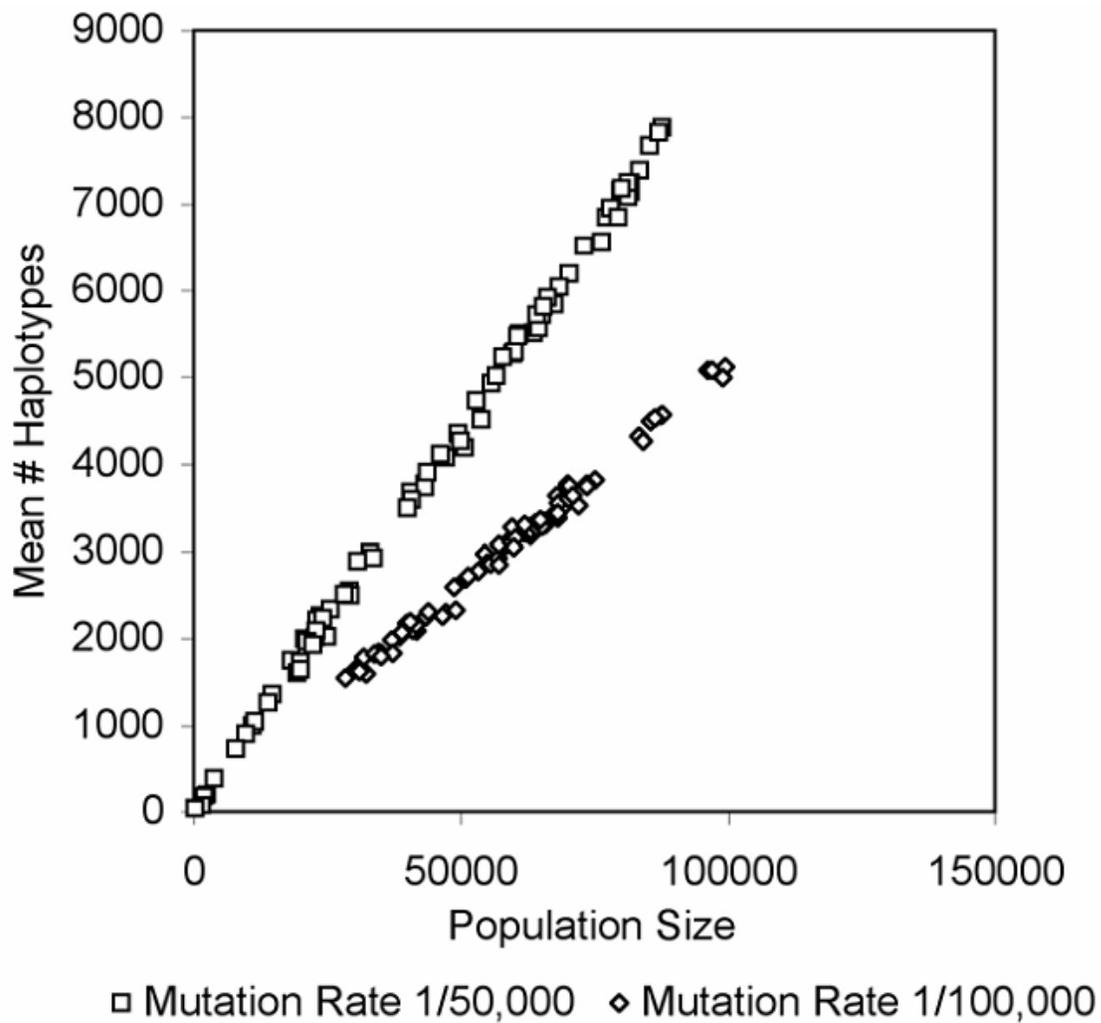
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Figure 1.2.5. Number of haplotypes sampled during simulations of neutral mutation with variable population sizes.

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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, we concluded that they have serious negative

implications for the use of mtDNA to infer cladistic relationships, places of origin, and

times of divergence. Aggarwal et al. (2003) argue based on very small samples that

Indian wolves are of ancient origin, suggesting 1-2 million years ago (mya). 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)? 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. 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."*

Templeton (1999) has criticized such arguments with the observation that mtDNA

diversity within Africa is higher in food-producing groups than in hunter-gatherers,

whom everyone believes came first. He argues (we think correctly) that increase in

genetic diversity in humans is more likely to be due to differences in population size and

growth rates than to population bottlenecks. More generally, these results may help to

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explain 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. 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 still exists. So far the geneticists' contribution to all this is to create problems, suggesting new species (Wilson et al. 2000) and hybrids between existing species. Kyle et al. 2006 wrestle hopelessly with both a genetic and a morphological solution to the question of species of eastern North American wolves. What the data showed were 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 clearly illustrate that these are subspecies or races within the genus.

Subspecies

The history of the trinomial nomenclature is probably more complicated and confusing than the history of the species concept. Mallet (2001) traces that history from Linnaeus to the present, discussing the different motivations for taxonomists to use a trinomial taxonomy (see also Mayr 1982).

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A subspecies is a non-random distribution of alleles, geographically based. A subspecies is a variety (breed, race) within the sexually isolated species, but not in itself sexually isolated from other subspecies. As we will show, all species that have a large range will have numerous subspecies.

It is assumed that the observable phenotypic variation in morphology, defined by the allelic distribution, is the result of local adaptations to topographic variation. The Darwinian (New Synthesis) belief is that varieties, races, or subspecies are incipient species and are on the path to becoming true and new species.

We think that could be correct, but it is an assumption and has by no means a guaranteed outcome. Sub-speciation as incipient speciation has been experimentally tested and never shown to work as expected. All experiments that selected for a change in allelic frequency never have produced a species. Richard Goldschmidt 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. He came to the conclusion that that *"the changes necessary for the formation of a new species are so large that the relatively small differences of the subspecies as a starting point would hardly count"* (Goldschmidt 1933, as cited in Gottlieb 1992).

Mayr (1982) suggests that sympatric speciation is conceptually difficult to postulate because of the lack of sexual isolation within a population. Simply put, this means that subspecies, which are in genetic contact (suture zones) with other members of its species, can never achieve complete sexual isolation. There are at least three examples of apparent sympatric speciation in the literature. The hawthorn and apple maggot flies

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are the best studied. They can be bred together in the lab but they have different life

cycles, coinciding with the flowering and fruiting times of the apple and hawthorn (Bush

1969). Berlocher and Feder (2002) review the data thoroughly and conclude, as with

many species arguments, *"One's perspective on this matter depends, in part, on one's*

concept of a species." The point is that authors treat populations that have some degree of

sexual isolation as if they were species.

This is not to say that on large continents, species could not be separated for

thousands of years, or that in some sense sympatric speciation could not happen, but

rather that it has not been clearly demonstrated. To us the emergence of dogs surrounded

by the ancestral population 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.

The Adaptive Subspecies: Clines

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 north 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.

It is assumed that this gradation of surface to volume ratios is indicative of an

adaptive response to latitudinal climate gradations. There are often other morphological

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changes associated with changes in radiative surface areas such as length of appendages and ear lengths. Phenotypic variations from north to south support theories describing clinal distributions within a single species. For a detailed analysis of the clinal "speciation" see Geist (1987). There are other kinds of clines, which involve altitude changes or edaphic variations over the species range.

In theory, genes can "flow" from one end of the cline to the other. It may be possible to show experimentally in some cases that individuals chosen artificially from either end of a cline are not inter-fertile. In the literature this is commonly referred to as ring speciation. But often the infertility is the result of mechanical problems related to size, or physiological responses to seasonality that trigger timing of oestrous cycles or spermatogenesis. Many other clinal differences can be developmental rather than genetic, such as variation in dialect of species song or courtship performances related to species recognition patterns (West-Eberhard 2003). Within clinal and ring speciation complexes many of the morphological and physiological differences may be developmental characteristics and not necessarily indicative of genetic differences.

Rarely are clines perfectly continuous over the niche, and rarely do they perfectly follow geomorphic features such as latitude. See Geist (1987) for an interesting review and a criticism of Bergmann's rule.

Frequently, regional morphological variants have historically acquired species binomials. This was especially true where there is some allometric growth characteristic. For example, bigger sheep have disproportionately larger horns and curling characteristics than their clinal relatives, and bigger deer have disproportionately larger

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antlers. Geist (1971) tried to deal with the clinal distribution of sheep, which range from African small-horned mouflons, across Asia to the bighorn sheep of Siberia and Alaska. Like the genus *Canis*, all members of the sheep cline (*Ovis*) are inter-fertile. The wild forms are also dwarfed in numbers by domestic sheep, which can interbreed with them and spread diseases among them. Geist argues that so many "species," "races" and "subspecies" of the genus *Ovis* have been named that one is hard-pressed even to remember the names. In the past, biologists like Geist "looked the other way" when it came to the species or subspecies designation.

Similarly, each of the 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. It would be difficult for us to think of the Ethiopian wolf or the red wolf 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. Indeed the evidence is just the opposite.

Shifting Clines

The grading from large polar morphs to small equatorial morphs is not perfectly linear. As the glaciers retreated rapidly 15,000 years ago, the tundra ecozone moved north. With the movement of the tundra went the tundra-adapted ecomorphs, the larger members of the cline. The temperate ecomorphs and the subtropical ecomorphs also

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"slid" north. Sliding north can mean populations of animals dispersed north with the

warming trends or that adaptation selected for smaller individuals *in situ*. Geist (1987)

developed his "dispersal hypothesis" in light of radiation to new luxury environments and

re-adaptation to old niches being a major feature of Ice Age mammal speciation.

The *Canis* cline did not shift north uniformly, just as the glaciers did not

disintegrate perfectly along latitudinal lines. Neither did the various size classes of *Canis*

shift in synchrony with the retreating glacier. There were discontinuities of movement in

every taxa associated with glacial retreat. Currently, the vegetation of Alabama, Georgia,

South Carolina, and North Carolina has relic populations of eastern hemlocks (*Tsuga*

canadensis). Hemlock is now most populous in areas that were under the ice 10,000 years

ago. These trees in isolated populations are classified as relic populations.

Glacial retreat is punctuated by numerous extinctions, clinal shifts, temporary

refugia of relic populations, and selective adaptations of the survivors. Stranded *Canis*

populations readapt to the new climate—if they have time, or get out-competed by the

smaller morphs of their own species moving north with the warming climate. All these

changes are happening millimetre by millimetre over hundreds or even thousands of

years.

Although the larger members of the genus 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

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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.

Clines are a good *a posteriori* argument for adaptive responses within a species whose niche covers a large continental area. The size of the species can vary regionally with not only the climate, but also the prey base, which in itself varies climatically and regionally. Again, this does not mean these regional variations are criteria for speciation. In addition, as we have seen, these clines may be disrupted and fragmented frequently by changes in climate, food supply, and countless other variables.

Non-adaptive Mechanisms of Subspecies

Our exploration of the subspecies question leads us to interesting findings. Traditionally it was thought that subspecies were locally adapted—a locally evolved incipient species. This may be true for many geographical variants but our studies suggest that there are non-adaptive methods in place that guarantee the creation and recreation of sub-specific variants. The result is that not all observable variation was "selected for."

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Subspecies are defined above as a non-random distribution of alleles, geographically based. The assumption is that this non-random distribution is the product of natural selection. However, there is a possibility—perhaps a probability—that allelic differences can be distributed non-randomly across the niche by local founder effects.

Populations are never constant, their numbers rising and falling sometimes rapidly because of disease, parasites, or other cataclysmic events. After such an event, the population recovers regionally from small founding populations. Thus, one would expect local founder effects. Theory predicts that a local founding population could not represent the gene distribution of the parent population.

Founder effects in geographically dispersed populations can be illustrated with simple simulations. To demonstrate this we designed a simulation in which we model 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.

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

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"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

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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 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 localization number that ranges from 0 to 1. A localization of 0 means that all genotypes occur everywhere across the table top, while a localization of 1 means that
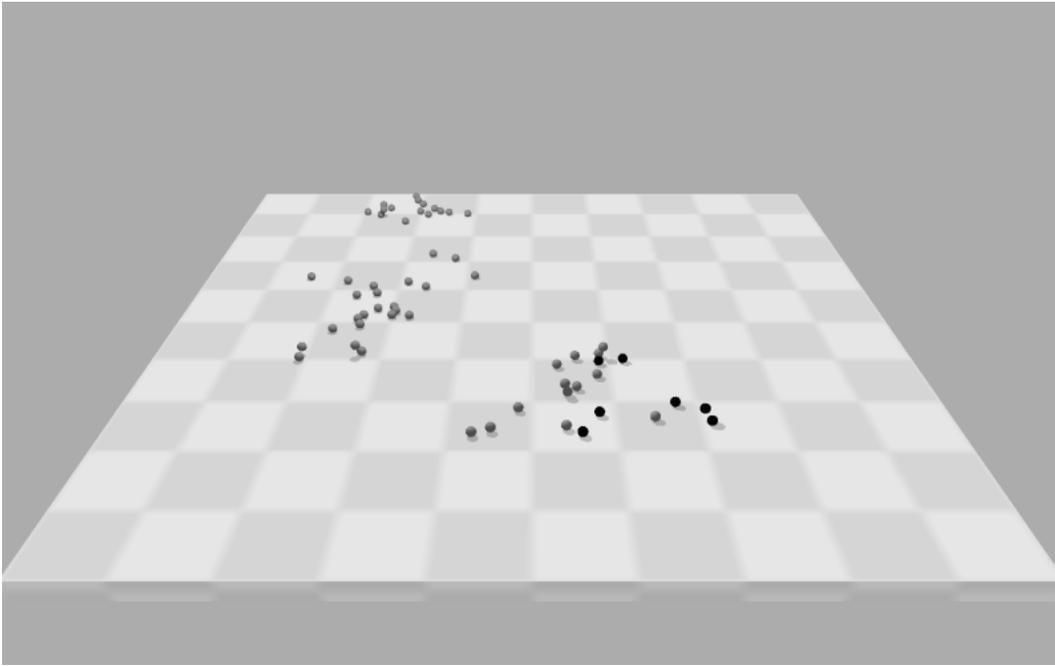
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Figure 1.2.6. Early snapshot of a "virtual marble" simulation demonstrating founder

effects, with colours reduced to shades of gray.
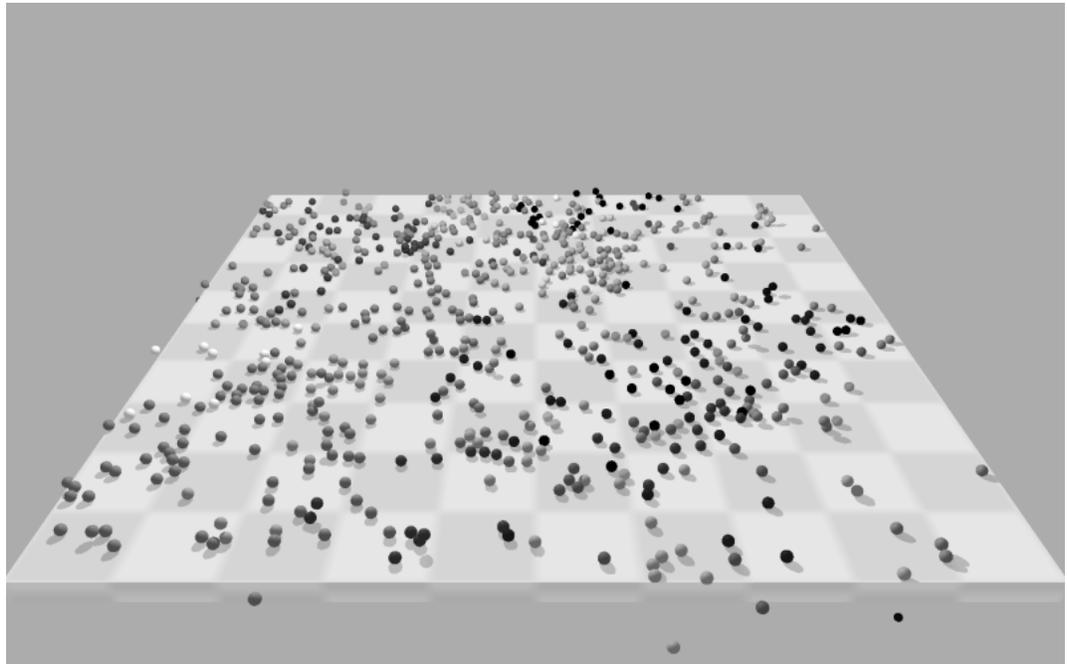
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Figure 1.2.7. Later snapshot of a "virtual marble" simulation demonstrating founder effects, with colours reduced to shades of gray.

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each genotype occurs only in one grid square. In general, the localization can be thought of as the percentage of the territory in which a typical marble's genotype will **not** be found.

Our primary observation from running these simulations was that geographically distinct subspecies readily emerged, even without adaptive selection of any sort. The environment was homogeneous and all the genetic material was selectively neutral, but mutation and founder effects nonetheless produced genotypes that varied across the territory, thereby warranting classification as subspecies. The patterns of sub-speciation depended on the values of several parameters, for example the mutation rate and the travel speed of the marbles, but significant sub-speciation occurred in a wide range of settings.

We plotted the localization 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 never got very low and it rebounded after about 3,000 time steps. At the end of each simulation the approximately 700 marbles were fairly well segregated geographically, with most marbles belonging to subspecies that covered only about 1/4 of the tabletop. We also plotted the numbers of subspecies in the same simulations (Fig. 1.2.9). Note that the hybridization condition produced more subspecies while maintaining approximately the same level of localization.
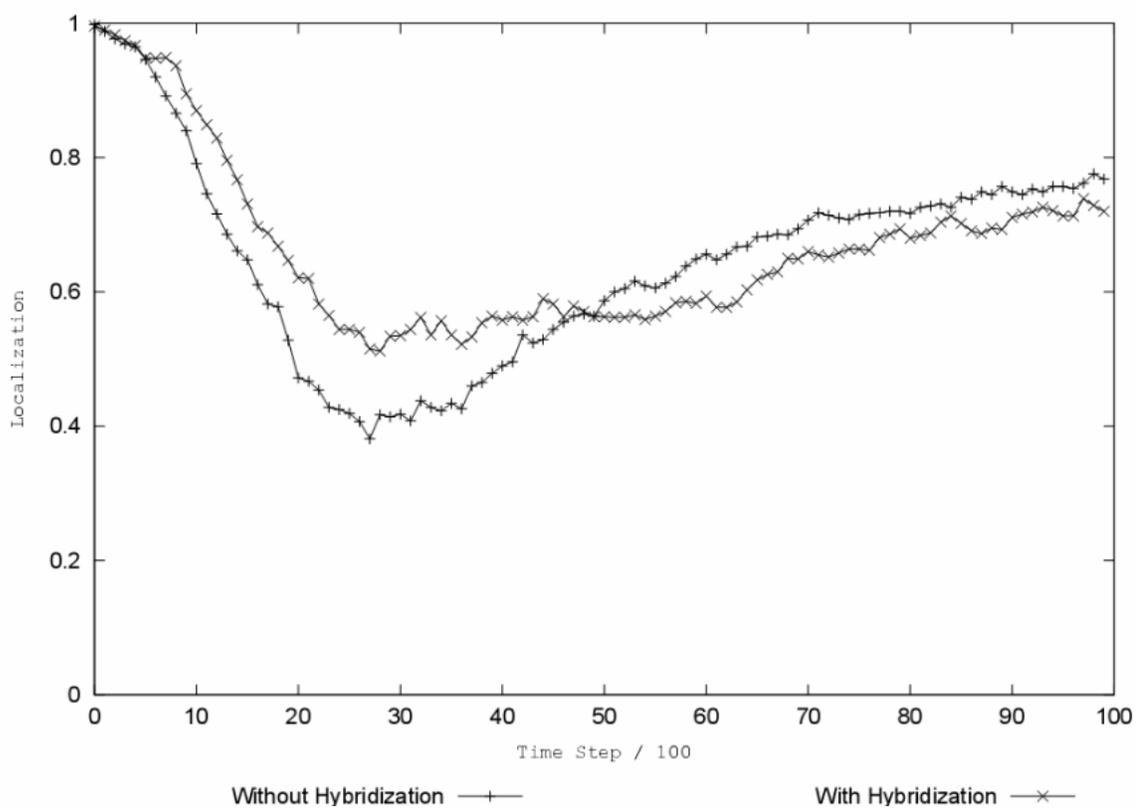
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Figure 1.2.8. Localization values for ten "virtual marble" simulations (five with

hybridization, averaged together, and five without hybridization, averaged together).
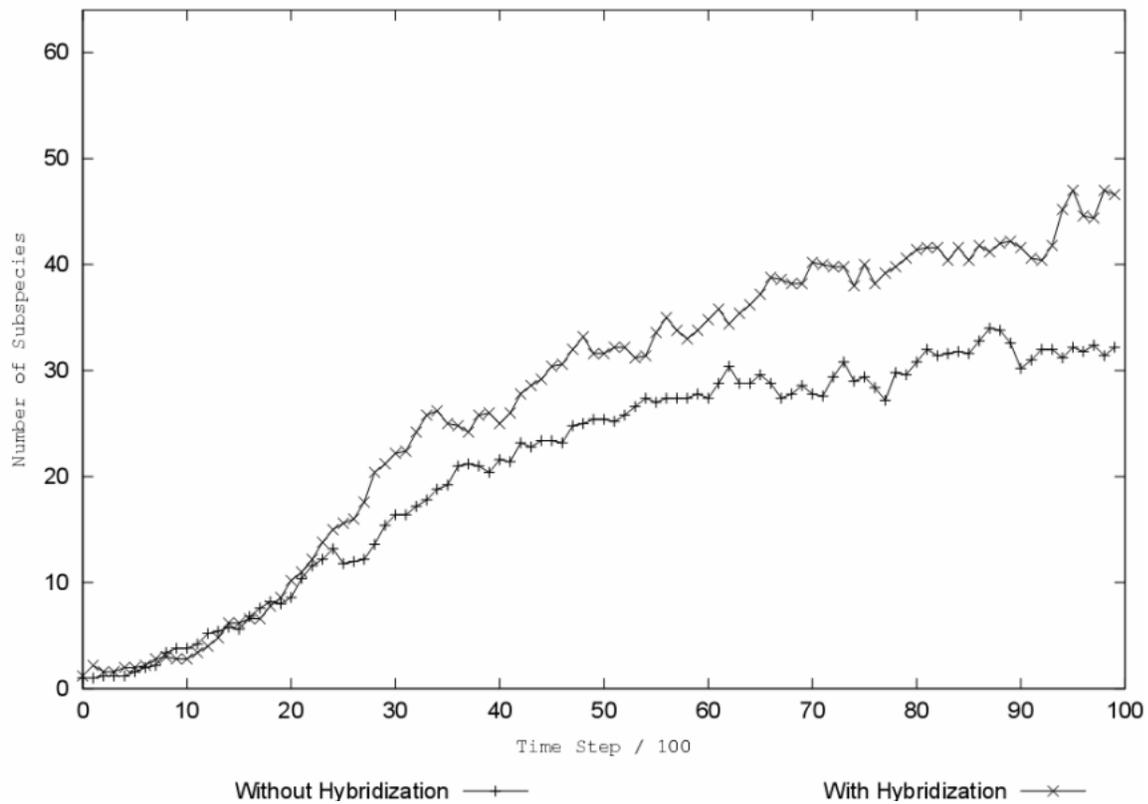
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Figure 1.2.9. Numbers of subspecies for ten "virtual marble" simulations (five with

hybridization, averaged together, and five without hybridization, averaged together).

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Correct interpretation of the localization measure depended additionally on the distribution of genotype population sizes. If all the groups with identical genotypes were approximately the same size, then the numbers presented above would not necessarily reflect geographically based subspecies; this is because each group would then be so small that it would count as well-localized even if its members were scattered across the tabletop. However, the distributions that we observed were far from uniform. In typical runs, 50% of the marbles fell in just 15% of the groups, and 90% of the marbles fell in the largest 50% of the groups. Most of the groups were small and therefore contributed little to the overall population localization. So the high localization numbers mean that even the large groups of single genotypes were restricted in geographic range.

The model illustrates why there can be so many subspecies represented in any population of animals with worldwide distributions. 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). In addition, it is likely with cataclysms that both re-population from the periphery and founder effects will operate simultaneously.

Rigorous selection for some diagnostic characteristic such as size does not preclude rapid and continuous sub-speciation in more selectively neutral characteristics. This model would predict that if any mtDNA haplotype would be distributed non-randomly throughout the range, it then would appear as a sub-specific characteristic. Sub-specific distribution of mtDNA would always appear in some cladistic representation of a

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species. The fact that these different haplotypes are neutral to selection suggests that local founder effects determine their distribution.

If within a cline we get a disease, the local population can run to zero. If it does locally go to zero then repopulation will come from the periphery. The area will be repopulated by "subspecies" from adjacent areas—maybe more than one subspecies—and hybridization of sub-specific varieties will necessarily occur.

It is important to note that centers of heterozygosity or local homozygosity (subspecies) are typical of local fluctuating populations and not indicative of either cladistic relationships or centers of origin.

Hybridization

"…[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."
"When they have arisen, they must justify their existence before the tribunal of 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, especially in rapidly radiating groups."

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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). It may be that the genus *Canis* is a polyploidy result of some fox-like ancestor. 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 agricultural sense of the word hybridization is often used in production of new "breeds" and characteristics such as hybrid vigour. The point is that when biologists typically thought of the hybrid being maladapted it was because the change in karyotype sexually isolated the population and in whatever landscape it became the "hopeful monster." 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.

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Coyotes breeding with gray wolves (Mech 1970), and coyotes breeding with red wolves (Nowak & Paradisio 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. Indeed, it is more likely that the three wolves in Eastern Europe had dog mtDNA. (Would it be fair to say that wolves descended from dogs?) 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 given to them. The researchers "know" what the species is before the research begins. They label the specimen collected a wolf, a coyote, or a dog, before the genetics is determined. The specimen has been identified before we send the tissue to the laboratory. We send a wolf sample to the laboratory, which then reports that the mtDNA is not wolf mtDNA, and therefore our wolf is a hybrid. Our wolf is carrying

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coyote mtDNA. Our wolf is carrying dog mtDNA. Our red wolf looks like a red wolf but it really is a hybrid coyote in disguise.

In each case it is assumed that sharing haplotypes is evidence of hybridization of the "species." In each case it is assumed that Linnaeus and his followers were right in designating these different species in the first place. In each case it is assumed that the morphology indicates species and in each case it is assumed that the genetic techniques can discriminate between those "species."

There are two 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. As in our diagram on skull shapes, cladistic mitochondrial relationships cannot be evidence of phylogenetic relationships, but rather of local founder effects.

The thought of hybridization brings out a prejudice in the adherents of Linnaeus's binomial system. Species in a Darwinian system must have monophyletic origins. 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

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managers will be able to sample the recovering population and weed out any red wolf offspring that show coyote or dog mtDNA. The intent, then, of the U.S. Fish and Wildlife Service is to create a canid-free zone around pure red wolves to prevent hybridization. A similar approach has been proposed for the Ethiopian highlands, to neuter local dogs and (hopefully) reduce the flow of genes to the Ethiopian wolves (Laurenson et al. 1997). It sounds like purebred dog breeding.

There is a growing literature that suggests that hybridization might be a—and possibly the—major source of genetic variation. 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..."* Coppinger and Coppinger (2001) suggest that hybridization is a way to create forms that are phylogenetically bizarre, what others have termed hopeful

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monsters. 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 "bad" or irrelevant to evolution: *"...natural hybridization affects the evolutionary history of the groups in which it occurs primarily through the production of novel genotypes [phenotypes] that in turn lead to adaptive evolution and/or the production of new lineages."*

Arons and Shoemaker (1992) demonstrate the idea of novel phenotype as a product of hybridization. In a study of neurotransmitter patterning of the midbrain stem they produced data demonstrating that hybrids are seldom the average of their parents but often have unique brain maps. The embryologists Alberch and Alberch (1981; Alberch 1982) demonstrate a number of threshold effects in the digital formula by changing the size (number of cells of the limb bud) of the organism. The argument that hybridization is a way of creating novel genotypes and corresponding phenotypic differences might be a surprise to canid biologists, but it is quite an old-fashioned idea for botanists (Mallet 2007).

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. Those of us who have studied hybridization are of the opinion that it increases the individual's fitness and creates novel

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behaviour patterns, which allow the exploration and novel adaptation to new habitats

(Arnold 1997; Doolittle 2005). Since hybridizing processes are occurring naturally in the

genus *Canis,* it would be 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).

Indeed, it may be the strength of any genus not to speciate in the sense of sexual

isolation. Habitats are always changing, and populations have to adapt continually.

Populations of canids are constantly faced with disease and other calamities. Sexually

isolated species will have fewer genetic capabilities to survive and adapt continually from

small populations. The ability of small populations to gather new genes by hybridizing

must be considered a selective advantage.

Phylogenetic Web

Microbiologists who are also interested in phylogeny have been frustrated in their

attempts to trace phylogenetic trees because of the high rate that their organism swaps

genes between species. There are essentially two methods of doing this, which Doolittle

(2005) labels lateral gene transfer (LGT) and homologous recombination.

Once one realizes that existing "species" can swap genetic material and use that

material in adaptive ways, the idea of a phylogenetic tree becomes obsolete. 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

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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). The

morphometrician tries to make sense out of size differences or colour differences. The

geneticist tries to analyze their DNA, based on the assumption that they are monophyletic

species.

And they are not monophyletic species (Fig. 1.2.10). Geneticists have provided

the data to prove it.

Conclusions

The genus *Canis* presently is divided into eight species (or seven, depending who

is counting). All were named before Darwin's theory of evolution. For nineteenth century

naturalists to declare some population of animals a species does not make those animals a

species. 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 are not indicators of

species differences. No physiological method has ever distinguished among them. The

only thing genetic studies have shown clearly is that there is a gene flow between existing

populations. They have never been demonstrated to be separate species.

Every paper that tries to deal with speciation in *Canis* claims a problem with the species

definition. There is no problem with the species definition. A species is a sexually

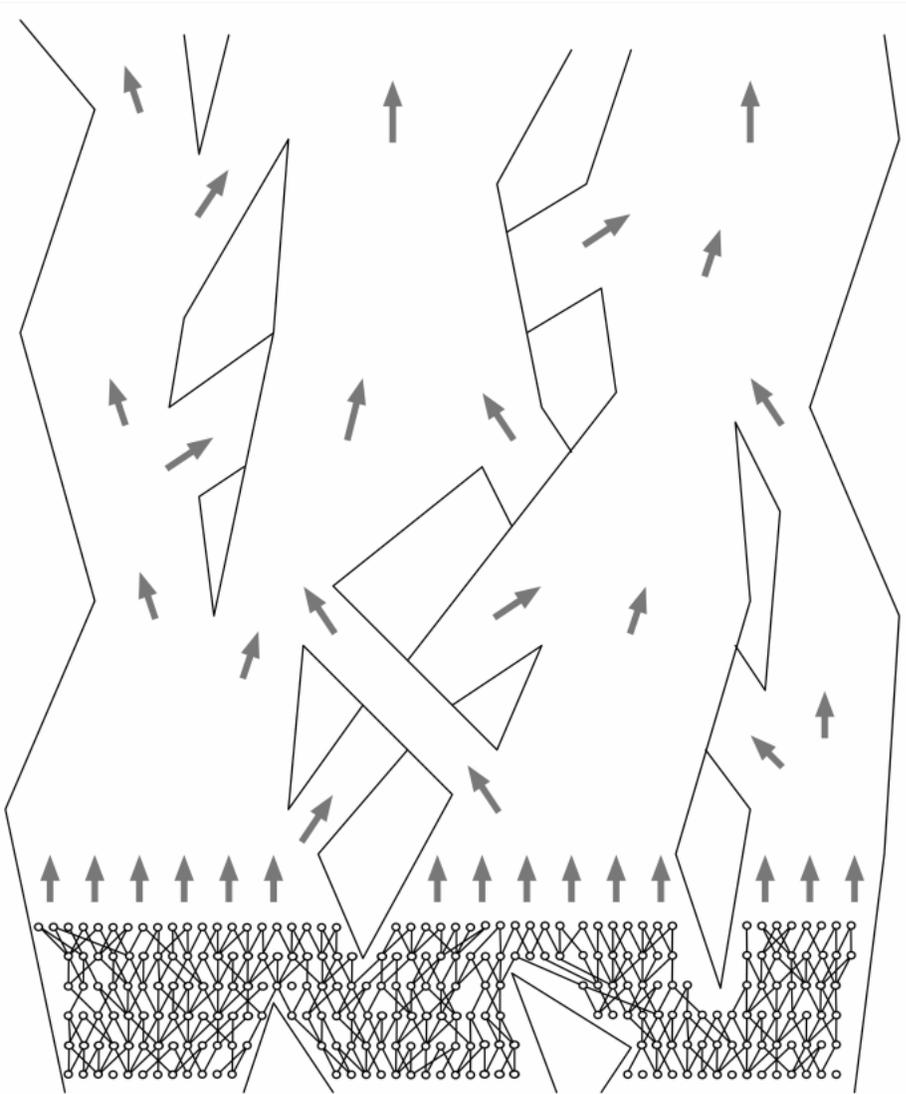isolated population. The problem with following the definition is historic precedent

Figure 1.2.10. Schematic diagram of the phylogeny of the genus Canis.

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combined with competing but inadequate methodologies.

From the point of view of conservation management, *Canids* should be recognized as subspecies. Restoration programs should encourage viable populations of the genus *Canis* in the habitats they wish to restore.

If there is still the wish to call these populations wolves, or red wolves, or Algonquin wolves, for popular or political reasons, then so be it. Call them anything that will expedite management. In the American ESA, subspecies are protected.

Both hybridization and change are normal among wild populations. Hybridization is very normal, an indistinguishable in sub-specific populations. Indeed hybrids are a subspecies by definition and should not be discriminated against in conservation programs. This is what the sophisticated new methodologies using mtDNA and cladistic statistics are telling us. Gene flow is constant. "Pure" species are wishful thinking. Hybridization increases genetic variability and in some instances creates phenotypic novelties. Hybridization should not be artificially prevented in reintroduction 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 eight (or is it seven) species of the genus *Canis* in the world are the results of

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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.

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