ARTIFICIAL SELECTION: A POWERFUL TOOL FOR ECOLOGISTS

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Abstract. Artificial selection has been practiced by humans since the dawn of agriculture, but only recently have evolutionary ecologists turned to this tool to understand nature. To perform artificial selection, the phenotypic trait of interest is measured on a population, and the individuals with the most extreme phenotypic values are bred to produce the next generation. The change in the mean of the selected trait across each generation is the response to selection, and other traits can also evolve due to genetic correlations with the selected trait. Artificial selection can directly answer the question of how quickly a trait will evolve under a given strength of selection. This kind of result can help ecologists determine whether range or niche boundaries are determined by a lack of variation for a key phenotypic trait or trade-offs due to genetic correlations with other fitness-related traits. In a related approach, controlled natural selection, the organisms are not selected according to their values for a given trait, but rather are allowed to evolve for one to several generations under experimentally imposed environmental treatments such as temperature, light, nutrients, presence or absence of predators or competitors, etc. The results of this kind of study can tell us how quickly a population can adapt to a given environmental change, either natural or anthropogenic. Finally, artificial selection can create more variation for measurements of natural selection or can be coupled with QTL mapping; both these combinations provide new insights into adaptation. I discuss advantages and disadvantages of these approaches relative to other kinds of studies and highlight case studies showing how these tools can answer a wide range of basic and applied questions in ecology, ranging from niche and range boundaries and character displacement to climate change and invasive species.

Key words: adaptation; artificial selection; character displacement; constraints; controlled natural selection; environmental change; genetic variation and correlation; niche and range boundaries.

INTRODUCTION

All of our domesticated plants and animals, from crops to pets, from Chihuahuas to Great Danes, are the products of artificial selection, Darwin devoted the entire first chapter of *On the Origin of Species* to the products of artificial selection, in particular the morphologically diverse breeds of domesticated pigeons. Artificial selection is simply human-directed evolution, and is important to biologists because it tells us what a given strength and form of selection can accomplish in terms of phenotypic change and how quickly this change can occur. In other words, artificial selection is the best technique to determine the nature and strength of genetic constraints on evolutionary change.

Why are evolutionary change and genetic constraints important to ecology? Antonovics (1976) made a strong case for why ecologists should care about genetics, and two of his ''tenets'' are directly relevant to artificial selection. One states that, because the range of environments that an organism can inhabit may be determined at least in part by genetic constraints, explaining

the distribution and abundance of species is partly a genetic issue. These constraints include lack of genetic variance for a trait, or a genetic correlation between traits that are both under natural selection. Through artificial selection the ecologist can test the ability of a species to adapt to conditions outside the boundaries of its niche or geographic range.

Antonovics also claimed that the distinction between ecological and evolutionary time was artificial and misleading, and supported his view mainly with examples of rapid evolution in response to anthropogenic change. Therefore, an understanding of how quickly organisms can adapt to environmental change is fundamental to trying to predict and explain alterations in community composition that may result from climate change, invasive species, or habitat destruction and fragmentation (e.g., Etterson and Shaw 2001). There are also accumulating examples of rapid evolution in response to natural changes in the environment, especially community shifts such as introductions or local extinctions of predators or competitors (e.g., Seeley 1986, Reznick et a1. 1997, Thompson 1998).

Artificial selection is a particularly useful tool in the study of adaptation to the environment, which is an ecological as well as an evolutionary process. If a trait is important for fitness, then the variants that are less

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well adapted have already been removed by selection, so that adaptation in current populations is hard to demonstrate (Grafen 1988). Artificial selection can be used to increase the variation, recreating some of the putatively ancestral phenotypes that are thought to have been eliminated by selection. The results of this selection can then be placed in the field to test the fitness effects of these ''new'' variants.

A new and particularly exciting approach is to combine artificial selection with QTL mapping to understand the genetic basis of character displacement and other selective mechanisms of population and species divergence (see Lexer et al. [2003] for a related approach). Here, artificial selection is used to simulate divergent or disruptive selection in nature, and then the newly divergent lines are crossed to create a mapping population. By mapping the genetic changes that have occurred during artificial selection, one can determine the number of genes causing the crucial early stages of divergence and begin to examine their effects. If crosses between replicated selection lines are mapped separately, then the repeatability of divergent evolution at the genetic level can be assessed. These kinds of data are critical for understanding the patterns and rates of rapid evolutionary responses to environmental change, both natural and anthropogenic.

Here, I review each of these applications of artificial selection as a tool for ecological research. I highlight selected examples in each case and suggest the kinds of questions and studies for which this approach can provide new insights.

DEFINITIONS

Artificial selection provides information about heritability and additive genetic variances, covariances, and correlations. The total phenotypic variance (V_P) in a population can be broken down into environmental variance (V_E), additive genetic variance (V_A), and two components of nonadditive genetic variance due to dominance and epistasis (V_D and V_I, respectively). Of these variance components, only additive variance is directly available for natural or artificial selection to act upon and cause evolutionary change in an outcrossing species. Additive variance is often represented as the heritability, defined as the proportion of phenotypic variance that is due to additive genetic causes (V_A/V_P).

I will use the terms additive genetic covariance and correlation (r_A) interchangeably in this review, because a correlation is just a standardized covariance. A genetic correlation between two phenotypic traits can be caused either by pleiotropy, in which one locus affects both traits, or by linkage disequilibrium, in which the two traits are affected by distinct gene loci, but some evolutionary force creates and maintains a nonrandom association between the alleles present at these loci (Falconer and Mackay 1996). Genetic correlations are important because natural or artificial selection on one

character causes an evolutionary change in a correlated neutral character, or alters the response to selection in a correlated character that is itself under direct selection. In other words, nonadaptive evolution of the second character can occur due to genes shared with the selected trait.

APPROACHES

There are two approaches to artificial selection that need to be distinguished (see Fry [2003] for additional discussion). In the traditional breeder's approach, the experimenter applies a known amount of selection to a single phenotypic trait by measuring the trait and breeding only those individuals with extreme values for the trait (Fig. 1). The difference between the mean of the entire measured population and that of the subset selected for breeding is called the selection differential (S). After one or several generations of this selection, the per generation change in the mean of this trait (the response to selection, R) is measured. The ratio of these gives the realized heritability: $h^2 = R/S$.

The higher the heritability, the more rapid the response to a given strength of selection (compare Fig. 1A and B). If selected lines differ in traits other than the one selected after selection, then this correlated response to selection is clear evidence of an additive genetic covariance between the selected trait and these other traits (Fig. 1C). I use the term artificial selection only for this traditional breeder's approach, and give a number of examples of its application to ecological questions below. This type of experiment provides direct estimates of the magnitude of genetic variation for a trait and the genetic covariance between the selected trait and other traits. Therefore, artificial selection is the best way to determine how fast a single trait will evolve with a given strength of selection.

The second approach can be called controlled natural selection, or more accurately, natural selection in a controlled environment. I call it natural selection because the experimenter does not decide which individuals survive and reproduce, as is done in artificial selection, but rather imposes some environmental treatment (e.g., several temperature, food, or light regimens; presence or absence of a predator or competitor) and lets the organisms reproduce in this environment for one to several generations. The organisms from the different treatments are then all placed in the same environment (a ''common garden'') or samples from all treatments are placed in each of the treatment environments (a ''reciprocal transplant'') and the traits of interest are measured. Differences in trait means among treatment groups measured in the same environment means that there is additive variance for the trait(s), and is a direct measure of the speed of the evolutionary response to the different environmental treatments. This is also a direct test of whether genetic constraints limit an organism's niche along the environmental dimension being manipulated. Examples of this approach
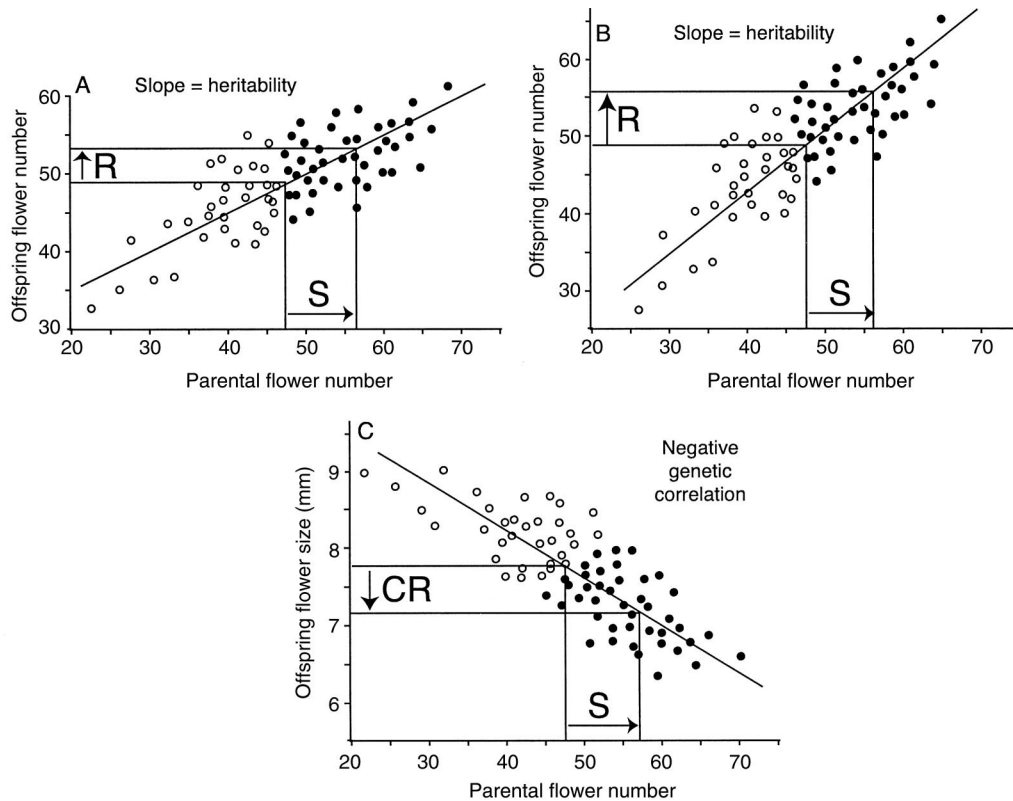
FIG. 1. Hypothetical data depicting heritability, response to selection, genetic correlation, and correlated response to selection. Each point represents mean phenotypic values for one full-sibling family. The lines drawn to the axes represent the means of the entire population before and after selection. (A, B) Regression of trait values (flower number in this example) in offspring on values of the same trait in the parents. The slope of this offspring–parent regression line is the heritability (h^2). The filled circles are the individuals selected for breeding; the difference in the mean of all parents and the selected parents is the selection differential, S. The difference in the mean of the entire parental generation (both selected and unselected individuals) and the mean of the offspring is the response to selection, R, which is the product of h^2 and S. The selection differential is the same in panels (A) and (B), so the greater response in panel (B) is due to the higher heritability (steeper slope). Note that, in a real artificial selection experiment, the offspring in the unselected families (open circles) would never be produced, because those parents would not be selected for mating, so the filled circles represent the entire offspring generation. (C) The relationship between one trait in the offspring and a different trait in the parents (flower number and size in this example) reflects the genetic correlation between these two traits. The genetic correlation is negative in this case, indicating a possible resource trade-off (cf. Worley and Barrett 2000). Selection to increase flower number in the parents produces a reduction in flower size (the correlated response or CR) in the offspring; the magnitude of the CR depends on the strength of selection and the magnitude of the genetic correlation between the two traits. Modified from Arnold (1987).

are also given in *Uses of Artificial Selection in Ecology: Controlled natural selection.*

Another approach is measuring the strength of natural selection in the wild, sometimes called "selection experiments." This term is both vague and misleading, because these studies are usually observational, not experimental, measuring the relationship between fitness and phenotypic variation in undisturbed populations. Unlike artificial selection, studies of natural selection in the wild are useful for understanding present-day adaptations, but they do not address questions concerning genetic variation or genetic constraints. Artificial selection imposes selection (S) to measure genetic variance (h^2), while studies of natural selection in the wild measure selection (S) without producing any information about genetic variance (h^2). Studies of

natural selection in the wild have been reviewed elsewhere (Endler 1986, Brodie et al. 1995, Kingsolver et al. 2001), and another paper in this issue (Lexer et al. 2003) discusses an extension to natural selection studies. Here, I address studies that combine artificial selection with natural selection, an integrated approach to studying adaptation and constraint.

USES OF ARTIFICIAL SELECTION IN ECOLOGY

Detecting genetic variation and covariation

Artificial selection is an excellent tool to determine whether a trait can respond to future selection due to natural or anthropogenic changes in the environment. It also allows testing of hypotheses about range or niche limits, especially when these hypotheses predict that

the range or niche of a species is limited by the values of a key phenotypic trait, such as the bill length of a hummingbird determining the species of plants the bird can feed on. By artificially selecting on that trait, the ecologist can determine if evolution outside the current range or niche is genetically possible.

Artificial selection has several advantages for determining genetic variation and covariation over the alternative methods of offspring–parent regression and sibling analysis (hereafter referred to as single-generation methods). First and foremost, artificial selection directly answers whether the trait can evolve in response to selection, whereas single-generation methods are indirect. Artificial selection is simpler conceptually and practically than sibling analysis, and has greater statistical power for the same number of individuals measured. This is primarily because artificial selection tests differences between line means, whereas sib analysis relies on variance and covariance components (Falconer and Mackay 1996). This means that artificial selection is a good choice for traits that are difficult to measure (e.g., physiology, behavior), because generally fewer individuals need to be measured at one time compared to single-generation methods.

Artificial selection also has some disadvantages compared to single-generation methods. Each artificial-selection experiment can measure additive variance only for the selected trait and can only estimate genetic covariances between this trait and other measured traits. Therefore, a single artificial selection experiment cannot be used to measure the entire matrix of genetic variances and covariances among a group of traits (G); a separate artificial selection experiment for each trait is necessary. In other words, artificial selection provides no information on the genetic covariance or correlation between two unselected traits, and does not provide a quantitative estimate of genetic variance or heritability for any unselected trait. This information is necessary for making quantitative predictions of the speed of evolutionary change for several traits (e.g., Campbell 1996).

Artificial-selection experiments are not practical for many organisms, because they typically require maintaining the organisms in the laboratory or greenhouse, sometimes for long periods, and controlled matings need to be performed. In some organisms, single-generation experiments can be done relatively easily in the field. In monogamous birds, for example, offspring–parent regression has often been carried out with natural matings in natural populations (e.g., Schluter and Smith 1986; reviewed in Boag and van Noordwijk 1987). Since the magnitude of genetic variance and covariance can be strongly affected by the environment (genotype by environment interaction), confining the experiments to unnatural environments can be a serious shortcoming if quantitative estimates of variance are of interest. A final practical problem is that artificial selection is most efficient if the individuals that are measured can then be mated, so that traits that cannot be measured on live individuals pose more difficulty. This difficulty can be overcome by measuring some individuals and using their clones (where possible) or full siblings to mate, but the latter will slow progress because the measured and mated individuals only share half of their genes.

Several studies have used artificial selection to demonstrate genetic variation for traits for which such variation might not be expected. These are traits that are maintained across higher taxonomic levels (families and above) and are diagnostic for these taxa. This means that ecologists should be wary of assuming a trait cannot evolve to adapt to new conditions simply because it varies little across species. Perhaps most remarkably, Holthorp (1944) selected for increased cotyledon number in *Brassica*, and was able to produce high frequencies of seedlings with three and four cotyledons after only two generations of selection. Therefore, a character that distinguishes dicots, and has been stable in some groups almost since the origin of flowering plants, is genetically variable. Two studies have found similar results at the family level. Huether (1968) was able to create populations of *Linanthus* with high frequencies of plants that deviated from the five petal lobes characteristic of the phlox family (Polemoniaceae). Dimorphic stamens within flowers (four long and two short) are diagnostic for the family Brassicaceae (mustards). Karoly and Conner (2000) reported significant decreases in dimorphism after one generation of artificial selection for decreased dimorphism in *Brassica rapa*, and after three generations a number of plants in the selected lines (but none in the control lines) had nearly lost the dimorphism (Fig. 2). Taken together, the results of these three studies suggest that a lack of additive genetic variation may rarely be the explanation for the evolutionary stasis of traits diagnostic at the family or higher taxonomic level. These traits may be maintained by stabilizing selection or constrained by genetic correlations with other traits under selection.

Artificial selection has also been used to test for the presence of genetic correlations, which are at the heart of theoretical work in a number of areas important to ecology, such as sexual selection, evolutionary constraints, and trade-offs among fitness-related traits. The eye stalks of stalk-eyed flies have been enormously exaggerated by sexual selection, so that the span between the eyes is greater than the overall body length in some species. Wilkinson (1993) reported a significant response to artificial selection for increased and decreased eye span in *Cyrtodiopsis dalmanni*, indicating that further increases in eye span are not constrained by a lack of variation. Females in these lines showed a correlated response to this selection; females in control and increased eye-span lines preferred males with larger eye spans, while females in decreased eye-span lines preferred males with smaller spans (Wilkin-
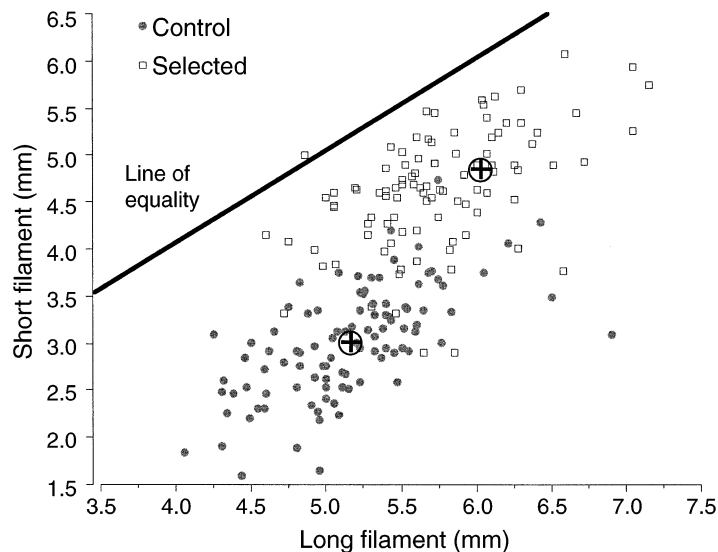
Fig. 2. Results of three generations of selection to reduce the dimorphism in anther heights, a diagnostic trait for the family Brassicaceae, in *Brassica rapa*. Note that a number of individuals in the selected line were at or near the line of equality, which represents no dimorphism (i.e., six equal-length anthers). Bivariate means for each of the two lines are indicated by the circled plus signs. Reprinted, with permission, from Karoly and Conner (2000).

son and Reillo 1994). This result supports a key prediction of some sexual selection theories—that sexually selected traits in males and the female preferences for them should be genetically correlated.

Negative genetic correlations have been sought as evidence for trade-offs between fitness-related traits using artificial selection. Siemens and Mitchell-Olds (1998) selected for increased and decreased constitutive levels of defensive chemicals (glucosinolates) and an enzyme (myrosinase) involved in the hydrolysis of glucosinolates into compounds that are more toxic. They found little evidence for the negative genetic correlations between constitutive and induced levels of defensive compounds that is predicted by allocation theory. They did report a negative genetic correlation between high constitutive levels of myrosinase and field seed production, evidence for a cost of defense. Using the same myrosinase selection lines, Strauss et al. (1999) reported one positive and one negative genetic correlation between myrosinase and floral traits affecting pollination, but for the most part pollination was uncorrelated with myrosinase. Worley and Barrett (2000) selected for increased flower number and both increased and decreased flower size (two replicates of each), and found significant correlated responses in three of the six total replicates; these were all negative, supporting the existence of trade-offs between flower size and number.

The studies described above performed directional selection on one or two traits to test for the presence of a genetic correlation between them. Another type of study selects on variation in two traits jointly, an approach called index selection by breeders. At least three studies have selected in a direction perpendicular to the major axis of a correlation between two traits, which means selecting in the direction of least variation in bivariate space (Fig. 3A). J. Conner and K. Karoly (*unpublished data*) selected on the ratio of filament and

corolla tube lengths (anther exsertion), two traits that are highly genetically correlated in wild radish (Conner and Via 1993; see *Uses of Artificial Selection in Ecology: Creating novel phenotypic variation*). The high and low ratio lines diverged rapidly, but the correlation within lines did not change (Fig. 3B). A very similar result occurred when Emlen (1996) selected on the residual of the sigmoidal allometric relationship between horn and body size in a dung beetle (*Onthophagus acuminatus*). This selection caused the position of the allometric curve to shift to the left and the right in the divergent lines, but the shape of the allometric curves within each line did not change. Stanton and Young (1994) selected on the ratio of petal area and pollen production in wild radish, also highly genetically correlated traits, and also obtained a rapid response. They did not report the correlations within each selected line after selection, so it is not known if they were altered.

These three studies clearly demonstrate that the lower amount of variation perpendicular to a strong bivariate relationship between two traits is not a strong constraint on evolution of the relative magnitudes of the two traits. While the relative magnitudes of the two traits responded to selection, the correlations between the traits within selected lines did not. This latter fact is not surprising theoretically, because these experiments imposed directional selection perpendicular to the correlation, rather than selection directly on the correlation (correlational selection; Brodie 1992). Thus, relationships among traits (correlations, allometries) may be quite robust in the face of directional selection that changes the means of these traits, at least over a few generations. This is relevant to ecology because these correlations may be adaptations to the environment (see next paragraph), and because correlations may cause trade-offs that constrain niche or range expansion. However, combining the divergently selected lines does result in a lower correlation (more
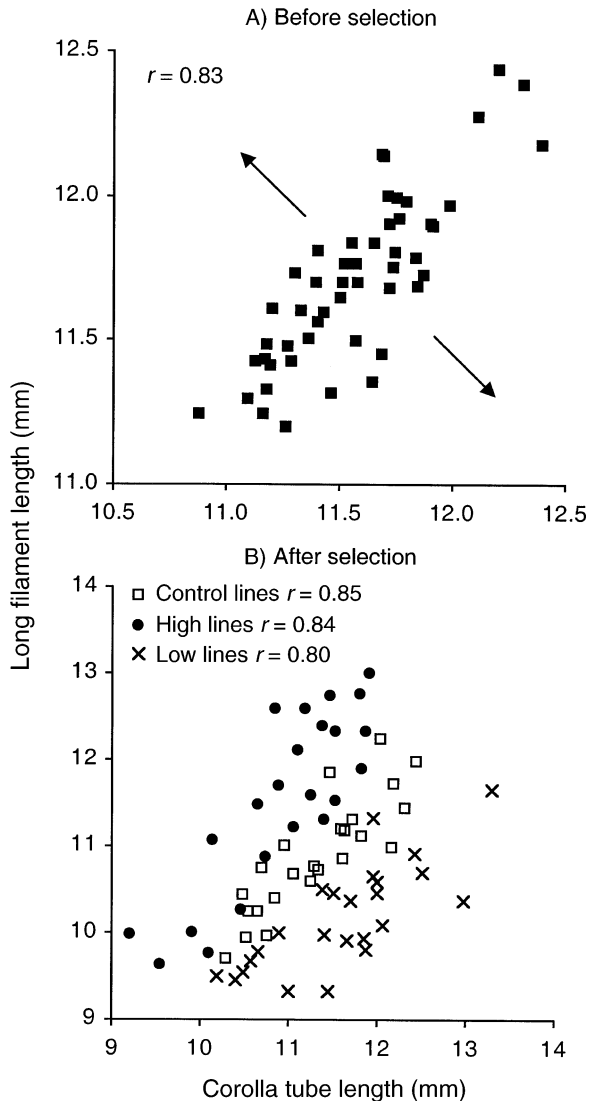
Fig. 3. Artificial selection perpendicular to the major axis of the correlation between filament and corolla tube lengths in wild radish (*Raphanus raphanistrum*). (A) The genetic correlation in the original population. The arrows show the direction of selection in the high and low anther exsertion lines; note that these are in the direction of least variation in bivariate space. Each point is the mean of all offspring of one sire from a nested half-sibling design (Conner and Via 1993). The correlation of these sire family means is an estimate of the additive genetic correlation. (B) Results after five or six generations of selection. Note that selection has moved the elliptical cloud of points in the directions of the arrows in A without changing the shape of the ellipse, that is, the correlation within each group. Each point is a full-sibling family mean; the resulting correlations are "broad-sense" genetic correlations that include covariance due to dominance and maternal effects. The difference in estimation methods between panels (A) and (B) is responsible for the greater range of values in panel (B).

variation perpendicular to the major axis) in the composite population in all three cases, a fact that can be useful for testing the adaptiveness of the correlation experimentally (see *Uses of Artificial Selection in Ecology: Creating novel phenotypic variation*).

Another feature that these three examples share is that the relationship between the two traits may be adaptive. The sigmoidal allometry between horn and body size in many horned beetles essentially gives rise to two morphs with differing mating strategies. Smaller males have little or no horns, and use stealth or other nonaggressive tactics to obtain mates, while large males use their horns in intermale combat (e.g., Eberhard 1982, Emlen 2000). Emlen (1996) suggests that divergence among species in the genus *Onthophagus* occurs by shifting the allometric relationship along the body size axis without major alterations in the relationship itself, similar to the response to artificial selection. In wild radish, the ratio of filament and corolla tube determines the position of the anthers relative to the opening of the tube (anther exsertion), and this affects pollination success and male fitness in this and other species (Conner et al. 1995, Morgan and Conner 2001 and references therein). Petal area in wild radish affects pollinator visitation frequency and thus pollen removal rates (Young and Stanton 1990, Stanton et al. 1991, Conner and Rush 1996), so that a correlation between petal area and pollen production could be adaptive. For example, a plant with large petals and low pollen production would have rapid pollen depletion, making further pollinator attraction a waste of the resources devoted to the large petals. Conversely, a plant with small petals and high pollen production might not be visited enough to have most of its pollen removed. Therefore, in all three cases the bivariate relationship between the traits found in natural populations may be adaptive.

Creating novel phenotypic variation

While artificial selection by itself sheds little light on adaptation, when used to generate increased variation for studies of natural selection, artificial selection can be an extremely useful tool for understanding the often difficult topic of adaptation. Ecologists commonly use observational methods of measuring natural selection in the wild (selection differentials and gradients; Lande and Arnold 1983) to attempt to understand which traits are adaptations to given biotic or abiotic factors in the environment. By using artificial selection to create additional variation for subsequent measurements of natural selection, one can avoid several problems associated with natural selection studies. The first problem is that the regression estimates of selection differentials and gradients have little power in the phenotypic extremes, because there are typically few individuals in the population expressing these extremes (Schluter 1988). This is particu1arly likely to be true if the trait is indeed an adaptation, because past
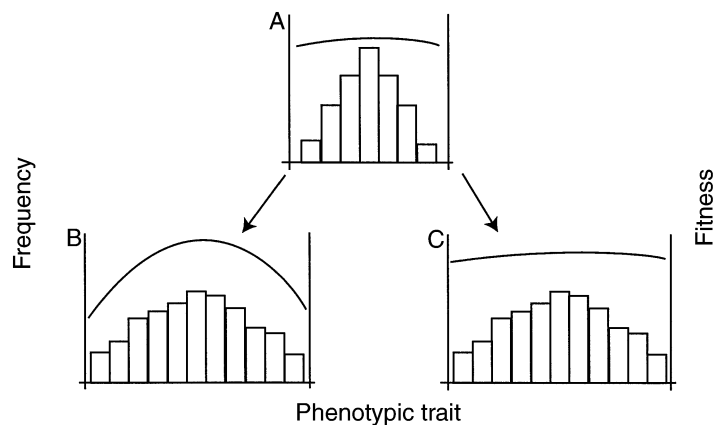
FIG. 4. A hypothetical example of the use of expanded phenotypic variation from artificial selection to test for adaptation. The histograms represent the phenotypic frequency distribution, and the curves above are the fitness functions showing the fitnesses of each phenotypic value. The fitness functions can be estimated as selection gradients. (A) The natural population; there is no significant selection, i.e., the slope and curvature of the fitness function are not statistically different from zero. (B) After artificial selection has increased phenotypic variance; there is now significant stabilizing selection, indicating that the trait is an adaptation. (C) Also after artificial selection has expanded variation. There is still no significant selection in this case, indicating that the trait is neutral (not an adaptation) over this expanded phenotypic range.

selection has eliminated extreme individuals. Artificial selection for extreme phenotypes can be very effective in increasing the representation of these extremes in the population.

A fundamental shortcoming of all studies of evolution in present-day populations is that the forces that created the trait of interest occurred in the past. While this can never be completely overcome, artificial selection combined with natural selection can help. As noted above, past selection may have eliminated unfit variants, so that a lack of selection in the present could be due to the current phenotypic distribution of the population occurring at an adaptive peak (Fig. 4A). Artificial selection cannot only increase the representation of existing extreme phenotypes, it can also increase the range of phenotypic variation; subsequent studies of natural selection on these artificially selected populations can determine if these new variants are less fit. If they are (Fig. 4B), then this is good evidence that the trait is adaptive. If the new variants are not less fit (Fig. 4C), then this suggests that the trait is neutral and thus not an adaptation. These kinds of data are even more interesting if combined with comparative or fossil information that indicates that these novel phenotypic variants were present in a recent ancestor.

A final problem with the observational techniques for measuring natural selection is that they can never prove a causal link between a trait and fitness, because there may be correlations between the trait of interest and unmeasured traits or environmental variables that are causing the appearance of selection on the trait (Mitchell-Olds and Shaw 1987, Rauscher 1992). For example, soil that is high in nitrates may cause plants growing there to have higher levels of alkaloid chemicals and higher fitness than plants growing in low-nitrate soils. An observational study would show positive selection on alkaloid levels, even if there was no causal link between alkaloids and fitness. These problems can be at least partly overcome by conducting divergent artificial selection on the trait of interest with replicate lines and random-mated controls. If selection in the field is then estimated in populations made up

of representatives of all these lines, then a significant difference among the mean fitnesses of these lines is powerful evidence of a causal relationship with fitness. Properly designed and conducted, this technique eliminates artifactual selection due to environmental and phenotypic correlations. However, traits that are genetically correlated with the selected trait will evolve under the artificial selection and thus could be the cause of fitness differences.

An alternative method to artificial selection for overcoming these same problems is direct manipulation of the phenotype, including artificial structures. For example, Andersson (1982) manipulated tail length in Widowbirds and measured the effect of this manipulation on pairing success, and Stone and Thompson (1994) and Schemske and Ågren (1995) created artificial flowers to test hypotheses of selection on anther position and flower size respectively. Direct manipulation can be much simpler and quicker than artificial selection, and can more easily create very extreme phenotypes. It also does not suffer from the problem of genetically correlated traits also evolving in response to artificial selection, which can cause interpretation errors. However, direct manipulation can have a similar problem, in that it can be very difficult to manipulate a trait without harmful or unknown effects on other traits. This can make it difficult to design appropriate controls, and may be an advantage for artificial selection because developmental constraints make it difficult for selection to produce large phenotypic changes, desired or not. In the extreme, there are some traits that cannot be directly manipulated without seriously harming the organism; similarly, there are some traits that are difficult or impossible to select on, or that will not respond to selection, although the latter seem to be rare. Therefore, often the choice between artificial selection and direct manipulation will depend on the organism and the specific trait of interest.

While artificial selection is more time consuming, in many cases it can produce a larger final sample size. For example, it is difficult to directly manipulate all the flowers in a population of a plant species that pro-

duces many flowers per individual. By artificially selecting on the floral trait and producing many seeds in the final generation of selection, very large sample sizes can be obtained at one time. Similar arguments could be made for insect species that can be reared in large numbers. On the other hand, artificial selection is more difficult than direct manipulation when the organisms are difficult to rear and breed in captivity. One final advantage of artificial selection over direct manipulation is that it produces information on genetic variation and constraint, topics that manipulation does not address.

J. Conner, K. Karoly, and colleagues are currently using artificial selection to test whether two anther position traits that lack variation at different taxonomic levels are adaptive. As noted in *Uses of Artificial Selection in Ecology: Detecting genetic variation and covariation,* dimorphic anther positions (resulting from stamen dimorphism) are present in most of the over 3000 species of Brassicaceae. Anther exsertion, the degree to which the long stamen anthers protrude beyond the opening of the corolla tube, has low variation in wild radish and some other mustards due to an extremely high correlation between the lengths of the stamens and corolla tube (Conner and Sterling 1995). Five generations of artificial selection for decreased dimorphism (analogous to the *Brassica* experiment in Karoly and Conner 2000), and for increased and decreased exsertion, produced increased variation for these traits in wild radish (e.g., Fig. 5). Note that the population with increased variance in exsertion is a composite of the high and low selected lines together with the random-mated controls. As discussed in *Uses of Artificial Selection in Ecology: Detecting genetic variation and covariation,* this selection did not alter the correlation between filament and corolla tube lengths within lines, and therefore did not alter the variance of exsertion within lines either.

These composite anther exsertion populations were placed in the field for natural pollination, and male fitness (seed siring success) will be measured when molecular genetic paternity analysis is completed. Two sets of complementary analyses will then be conducted. Analysis of variance with male fitness as the response variable and the selection treatment as the predictor variable will test the overall fitness effects of the treatments, correcting for phenotypic and environmental correlations (including correlations with unmeasured traits) but not for any correlated responses to the selection due to genetic correlations with the anther traits. Multivariate selection gradient analysis (Lande and Arnold 1983) will test for the effects of the selected traits on fitness, correcting for correlated responses in other measured traits but not unmeasured traits.

Controlled natural selection

As noted in *Approaches,* a closely related alternative to artificial selection is natural selection under con-
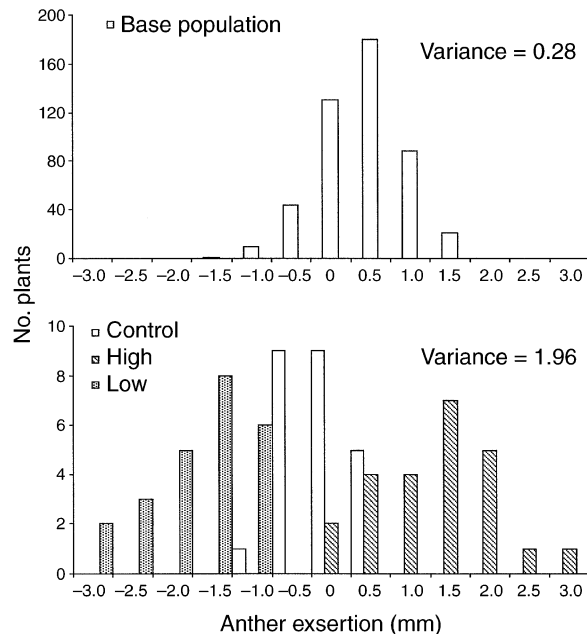

FIG. 5. Results of artificial selection for increased variation in wild radish anther exsertion. The top panel shows the base population from which the selection was started (N = 474), whereas the bottom panel shows the results of six or seven generations of selection (one set of three replicates was selected for a generation more than the other set; N = 72). Note that the variance within each selection treatment has not increased, but the variance of the composite population made up of the high, low, and control lines together is greatly increased relative to the base population. Data are from J. Conner and K. Karoly (*unpublished data*).

trolled environments, or ''controlled natural selection.'' Here, the experimenter manipulates an environmental variable and measures the evolutionary response. In this approach, selection is not directly imposed by the investigator as it is in artificial selection, but an environmental factor that may cause selection is manipulated. The results of a controlled natural-selection experiment show whether an organism can adapt to a specific environmental change, or move outside the boundaries of its current niche. Since a known amount of selection is not being applied to a specific trait, the heritability cannot be quantitatively estimated, but this is often not the primary concern to an ecologist. In addition, little or no information can be gained about genetic correlations among traits with controlled natural selection, because the traits that are the targets of direct selection are unknown. Fry (2003) gives examples of this approach applied to trade-offs.

Controlled natural selection can be conducted in the field with some species, if key environmental variables can be manipulated. An excellent example of this approach comes from work on guppy evolution in Trinidad (Reznick et al. 1997). In two river drainages, guppies were moved from high-predation sites with cichlid fish to low-predation sites above rapids or waterfalls

where there were no guppies or cichlids. After 7, 13, and 18 generations, samples of the guppies from the source (control) population and from the transplanted populations were brought to the lab. After one generation in the lab to reduce maternal effects, the age and size at maturity was measured. As predicted by life-history theory, in almost all cases the fish transplanted to the lower predation sites matured later and at a larger size than the high-predation control populations. This is a clear example of rapid evolution in response to a change in the environment.

Controlled natural selection can also be valuable to understand and predict evolution in response to anthropogenic changes in the environment, but this approach has rarely been used. In one of the best examples, Ward et al. (2000) selected for increased seed production for five generations in *Arabidopsis thaliana* at two concentrations of CO_2. Because seed production is an excellent measure of fitness for these selfing annuals, this is essentially a controlled natural selection experiment. The low CO_2 concentration mimicked the low levels that occurred in the Pleistocene, whereas the high concentration represented predicted levels by the end of this century. They found that plants flowered later and attained a higher biomass relative to random mated controls at low CO_2, and showed the opposite response in these traits at high CO_2. Ward et al. then performed a reciprocal transplant experiment with their selected lines, planting the last generation of each at both CO_2 concentrations, after first passing them all through a generation at intermediate CO_2 to minimize maternal effects (a critical consideration for all common-garden and reciprocal transplant studies). They found that both lines produced the most seed at the concentration they had been selected in, but the difference was only statistically significant when the plants were tested at the lower concentration. This latter result indicates that *A. thaliana* can rapidly adapt to decreases in CO_2, and perhaps to increases as well.

Controlled natural selection is therefore a valuable tool that could be applied to many more questions in ecology, particularly with short-lived organisms. Areas that seem particularly ripe for this approach are niche dimensions, range limits, and character displacement. By manipulating one or more dimensions of an organism's niche for several generations, one could test the environmental limits of adaptation of an organism, which could shed light on both niche and range limits. Placing individuals from allopatric populations of two species with broadly overlapping niches together for several generations might well reproduce the early stages of character displacement. This type of experiment would be an extension of a single-generation experiment demonstrating selection for character displacement in stickleback fish (Schluter 1994). The controlled natural selection approach could be used to predict the results of anthropogenic changes in the environment other than CO_2, such as habitat fragmentation,

invasive species, and biocontrol agents. For example, biocontrol agents have sometimes attacked native species related to the invasive they were imported to attack (e.g., Louda et al. 1997). Even if the biocontrol agent cannot survive on these native hosts when it is introduced, it is possible that rapid evolution of host range could occur after the introduction. This could be tested by rearing the biocontrol agent on different mixtures of the invasive and native species for several generations, to see if adaptation to, and use of, the native increases.

Future direction: combine with QTL mapping

I have discussed how artificial selection can be combined with studies of natural selection to produce new insights into adaptation. Another powerful combination is artificial selection (or controlled natural selection) and quantitative trait locus (QTL) mapping, which can be used to examine the genetic basis of adaptive divergence, including character displacement, local adaptation, and incipient speciation. QTL mapping uses genetic markers scattered throughout the genome to find gene regions that affect quantitative traits (Tanksley 1993, Mitchell-Olds 1995). Quantitative traits are those that are affected by several to many gene loci, as well as the environment, leading to a continuous phenotypic distribution. The vast majority of traits that ecologists are interested in are quantitative.

If QTL mapping is applied to a cross between divergently selected lines, then it can answer questions such as: How many gene loci were responsible for the observed response to selection? What is the distribution of the magnitude of effects of these loci on the selected trait, i.e., are there a few loci of major effect, many loci of small effect, or a continuum of magnitudes of effect? These genetic details matter to evolutionary ecologists, because they affect the direction and rate of adaptive evolution, and therefore affect interactions of organisms with their biotic and abiotic environments and the rate of adaptation to global change (Orr and Coyne 1992, Mitchell-Olds 1995, Orr 1998). This approach can also be used to probe the genetic basis of trade-offs, a central theme in evolutionary ecology (Fox et al. 2001). Artificial selection has already been used to study trade-offs (e.g., Service et al. 1988), and the addition of QTL mapping can greatly improve our understanding. For example, artificial selection combined with QTL mapping can tell us whether the trade-offs are due to pleiotropic loci, and provide a first step in determining what these loci code for. This knowledge would give us a mechanistic understanding of ecological trade-offs. Finally, if separate crosses between replicate divergent lines are mapped separately, then one can also ask whether evolution is repeatable; that is, are the same loci responsible for divergence in each case? This question may be relevant to the generation of biodiversity, as less diversity would be cre-

ated if evolution is highly repeatable than if evolution is more idiosyncratic.

Mackay and colleagues (Long et al. 1995, Gurganus et al. 1999, Nuzhdin et al. 1999) have conducted what is probably the best study combining artificial selection and QTL mapping for a natural population. They selected for increased and decreased abdominal and sternopleural bristle number in two separate sets of *Drosophila melanogaster* lines for 25 generations. They then produced detailed genetic maps from these selection lines, using several powerful genetic techniques that have been developed for this species. They found a total of 26 loci that were responsible for the response to their selection, and 20 of these mapped to locations of known bristle number or nervous system gene loci (the bristles are sensory). These results strongly suggest that these approaches can be successful in identifying the genes responsible for adaptation to specific factors in the environment, although this goal is more distant for nonmodel organisms.

A similar approach is being taken by J. Conner, L. A. Prather, and J. Hancock, using the wild radish anther exsertion selection lines. From each replicate pair of high- and low-exsertion lines, the plants with the highest and lowest exsertion were crossed, and then the F_1 offspring of these matings were crossed separately to create two separate F_2 mapping populations, one for each pair of high and low exsertion lines. QTL mapping will be used to identify the gene regions responsible for the rapid divergence of exsertion observed during the artificial selection. By mapping the crosses between the two replicates separately, we can ask how repeatable short-term evolution is genetically. Therefore, this kind of study should help ecologists understand the details of how adaptation to changes in the environment occurs, help to explain current species limits, as well as predict the speed of adaptation in response to natural and anthropogenic environmental change. Because wild radish is a serious weed, it may improve our knowledge of how weedy and invasive species can rapidly adapt to new environments.

Conclusions

In this review, I have tried to illustrate several ways in which artificial selection and controlled natural selection are useful tools for ecologists. These tools can provide unique insights into a number of fundamental and difficult issues, such as constraints on adaptation, trade-offs among fitness-related traits, niche and range limits, and character displacement. These multigeneration approaches have rarely, if ever, been applied to the latter two questions. When used to generate increased variation for studies of natural selection, or when combined with QTL mapping, artificial selection can shed light on the adaptiveness of traits in nature and the genetic mechanisms of rapid adaptation. Artificial selection and controlled natural selection can also be used to directly test the adaptive responses of

populations to a variety of anthropogenic changes in the environment. Knowledge gained from these kinds of studies will improve our ability to predict the effects of such changes on natural communities and agroecosystems. For these reasons, I argue that artificial selection and controlled natural selection should become standard approaches in ecological science.

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