Inter-locus antagonistic coevolution as an engine of speciation: Assessment with hemiclonal analysis

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One of Ernst Mayr’s legacies is the consensus that the allopatry model is the predominant mode of speciation in most sexually reproducing lineages. In this model, reproductive isolation develops as a pleiotropic byproduct of the genetic divergence that develops among physically isolated populations. Presently, there is no consensus concerning which, if any, evolutionary process is primarily responsible for driving the specific genetic divergence that leads to reproductive isolation. Here, we focus on the hypothesis that inter-locus antagonistic coevolution drives rapid genetic divergence among allopatric populations and thereby acts as an important “engine” of speciation. We assert that only data from studies of experimental evolution, rather than descriptive patterns of molecular evolution, can provide definitive evidence for this hypothesis. We describe and use an experimental approach, called hemiclonal analysis, that can be used in the Drosophila melanogaster laboratory model system to simultaneously screen nearly the entire genome for both standing genetic variation within a population and the net-selection gradient acting on the variation.

Hemiclonal analysis has four stages: (i) creation of a laboratory “island population”; (ii) cytogenetic cloning of nearly genome-wide haplotypes to construct hemiclones; (iii) measurement of additive genetic variation among hemiclones; and (iv) measurement of the selection gradient acting on phenotypic variation among hemiclones. We apply hemiclonal analysis to test the hypothesis that there is ongoing antagonistic coevolution between the sexes in the D. melanogaster laboratory model system and then discuss the relevance of this analysis to natural systems.

There is widespread agreement among evolutionary biologists that the allopatry model is responsible for generating much of the species diversity presently found within sexually reproducing lineages (1–7). Although substantial empirical evidence supports the operational steps of the basic allopatry model (8), there is no general consensus regarding the relative importance of alternative evolutionary processes that drive the specific genetic divergence that leads to reproductive isolation. Because allopatry necessitates that populations be physically separated, there can be no direct selection for reproductive isolation and therefore it must develop as a pleiotropic byproduct of the genetic differences that accrue due to the independent evolution of populations.

At a minimum, recurrent mutation and random genetic drift of neutral variation lead to genetic differentiation among allopatric populations. However, natural selection can greatly accelerate the rate of genetic divergence. Van Valen (9) used paleontological evidence to conclude that adaptation to the physical environment is asymptotic (declining in rate with time). Rapid adaptation occurs when a population initially experiences a new physical environment, but the rate of evolution slows as the population becomes progressively more adapted to the prevailing physical conditions. The rate of adaptation diminishes with time as the lag-load (the reduction in mean population fitness due to the average trait of a population differing from its optimal value) of the population decreases. In contrast, adaptation to the biotic environment is expected to be nonasymptotic when enemies (for example, predator and prey, host and pathogen, or resource competitors) become locked in a perpetual arms race of adaptation and counteradaptation. In this case of interspecific antagonistic coevolution, the lag-load of a species does not diminish with time because adaptive progress is continually eroded due to counterevolution by enemy species.

An analogous cycle of antagonistic coevolution can take place between genes that reside within the genome of a single species (inter-locus antagonistic coevolution). In this case, adaptive allelic replacement at one locus increases the lag-load at a second locus by generating selection for a new optimal allele, and the resulting adaptive replacement at the second locus in turn increases the lag-load at the first locus, thereby stimulating a new round of the antagonistic cycle of adaptation and counteradaptation. The genetic conflict that drives inter-locus antagonistic coevolution is termed “intragenomic” when conflict occurs between genes that reside in a single individual (for example, genetic conflict associated with genomic imprinting (10), meiotic drive (11), and/or cytonuclear conflict (12)) and “intergenomic” when gene products from different loci mediate conflicts of interest between different individuals of the same species (13).

Inter-locus antagonistic coevolution (intragenomic or intergenomic) can potentially drive rapid genetic divergence among allopatric populations because the antagonistic cycle of adaptation and counteradaptation maintains a persistent lag-load at each interacting locus and thereby drives perpetual evolutionary change. In this article, we focus on intergenomic conflict because two of its forms are predicted to contribute to reproductive isolation by causing genetic divergence among allopatric populations (13–16). Intergenomic conflict can occur both within and between the sexes. An example of intrasexual conflict occurs between gene loci that mediate the male “offense” and “defense” phenotypes in the context of male–male competition to fertilize eggs. A male that mates a virgin female is selected for a defense phenotype, that is, to prevent the female from mating with other males and to prevent his stored sperm from being displaced by a secondary male if the female remates. A male encountering a previously mated female is selected for an offense phenotype, that is, to induce the female to mate with him even if she has ample stored sperm from another male, and then to replace the stored sperm from the
Two arms races

Fig. 1. Inter-locus antagonistic coevolution within and between the sexes can promote the rapid evolution of reproductive traits that are expected to contribute to reproductive isolation (prezygotic mating isolation and postzygotic hybrid infertility) among allopatric populations.

The arms races between male offense and defense, and both of these processes with female resistance, would be expected to occur independently in allopatric populations and drive rapid genetic divergence among them (Fig. 1). Because, in principle, these arms races can be expected to cause changes in phenotypes influencing reproductive traits (mating behavior and reproductive physiology and anatomy), the genetic divergence produce by inter-locus intersexual arms race is expected to ensue between genes that promote female resistance to male-induced harm and the genes that code for male offense and defense (13–16).

The ability to predict the direction of evolutionary change requires that one establish that there is (i) standing additive genetic variation for a trait, and (ii) a non-zero net-fitness selection gradient on the trait in the prescribed direction. The ability to predict the direction of evolutionary change requires that one establish that there is (i) standing additive genetic variation for a trait, and (ii) a non-zero net-fitness selection gradient on the trait in the prescribed direction. Because of the recent, rapid advance of DNA-based molecular tools, our capacity to measure certain types of standing genetic variation in natural populations is no longer a limiting factor, for example, in genes with highly conserved regions that permit PCR primers to be readily constructed. However, measuring heritable standing genetic variation for fitness-related traits, and especially net-fitness itself, in natural populations remains a daunting task, although these traits can be approximated in special circumstances (see, for example, ref. 40). Although it is possible in the laboratory to accurately measure genetic variation and fitness components associated with many traits, the relevance of these measurements to net-fitness and heritability in nature is dubious. As a consequence, although we can presently measure the standing genetic variation for certain types of traits (such as
microsatellites) of natural populations with a high degree of accuracy, levels of heritable phenotypic variation, and the selection gradients on this variation, are unknown for most traits (Fig. 2). To circumvent this problem we have focused on the analysis of a locally adapted laboratory population of D. melanogaster. In this context, by cloning nearly entire genomes and amplifying them to large numbers, we can measure accurately both standing genetic variation for a trait and net-selection acting on this variation. Our purpose in this laboratory analysis is not to duplicate the natural history of wild populations, but to study the evolution of laboratory-adapted populations in their own right to deduce basic principles of evolution. Many laboratories throughout the world study laboratory populations; however, we describe here a particular form of evolutionary investigation that we call “hemiclonal analysis” that specifically applies to the D. melanogaster model system. Our approach has four stages that we describe and discuss in the sections below.

**Stage 1: Create a Laboratory Island Population**

The study of island populations has played an important role in the study of evolution, beginning with the pioneering studies of Charles Darwin and Alfred Wallace. It is our view that one of the major reasons that island populations have been particularly informative is that they are much simpler than continental populations (in the context of both biotic and abiotic factors) and therefore easier to understand. Laboratory populations represent island populations that conveniently reside within the laboratory where joint measurements of fitness and genetic variation are more feasible. They are far simpler than natural island populations, but this simplicity provides tractability in the context of evolutionary analysis. By comparison, ecologists have gained important insights into the extinction and colonization process by studying very small island populations of vertebrates in nature, despite the fact that these “islands” are little more than large protruding rocks, 1–16 m on a side (see, for example, ref. 41).

The utility of using laboratory populations to study evolution depends upon how much their evolution is regulated by the same principles that control the evolution of natural populations. Most past laboratory studies of D. melanogaster have used highly inbred stocks such as Oregon-R, Lausann-S, and Canton-S, or genetic samples that have been recently derived from nature and therefore have not adapted to the laboratory environment. In the latter case, the flies are tested in a novel environment so measures such as heritability and selection on the standing, heritable phenotypic variance are difficult to interpret. Inbred laboratory stocks lack these problems, but they have been bottlenecked many times, their extreme and uncontrolled crowding interferes with many forms of behavioral interactions that have historically been important in the species, and their laboratory culture varies among laboratories and stock centers. As a consequence, these inbred laboratory populations have evolved under crowded, uncontrolled conditions that preclude the importance of the rich repertoire of behavioral interactions within and between the sexes, and they have undoubtedly fixed for large numbers of deleterious alleles. To avoid these undesirable aspects of standard laboratory stocks, our laboratory, like others (e.g., the laboratories of Brian Charlesworth, Linda Partridge, and Michael Rose), has started a new laboratory population. The large outbred laboratory population that we study (LHM) was founded by Larry Harshman (now at the University of Nebraska) from 400 inbred females collected in an orchard near Modesto, California in 1991. Since then, it has been maintained at a large effective population size (>1,800 breeding adults). In 1995 the population density of juveniles and adults was reduced so that juvenile density was consistently maintained at between 150–200 individuals per vial, and adult density was reduced further to only 16 pairs per vial (placed on its side to allow more horizontal space for the flies to spread-out). The low adult density increased the potential for behavioral interactions to contribute the adult fitness of both sexes.

The population of flies pass through three sets of 56 10-dram vials during their 2-week generation cycle. On day 1, the eggs that were laid at the end of the previous generation are randomly reduced to 150–200 per vial to prevent the extreme crowding that occurs in mass-transfer laboratory cultures. The flies remain in these “juvenile competition” vials for 12 days during which larval competition, pupation, and the early adult stages occur. On day 12, the flies are mixed among vials and, after being randomly culled to 16 pairs per vial, are transferred to new “adult competition” vials where they reside for 48 h. Live yeast (10 mg) is applied to the top of the 10 ml of killed yeast medium in each vial. There is a steep linear relationship between the amount of live yeast applied and average female fecundity, indicating that live yeast is the major factor limiting female fecundity (42). In the adult competition vials, females compete intensely for the limited supply of live yeast, and males compete to inseminate females and fertilize their eggs. Eighteen hours before the end of the two-week generation cycle, the flies are transferred to “oviposition vials” (with no live yeast added), and the eggs laid at this time are used to begin the next generation. As a consequence, egg production in the oviposition vials represents the lifetime offspring production of both sexes. Put another way, the flies are selected to be “big-bang” reproducers, analogous to semelparous salmon. During the 2-week generation cycle, adults live for at most 6 days, and there is virtually no adult mortality during this time; however, larval mortality does occur in the juvenile competition vials at a rate of ~10% (43). In the following sections, all measurements of lifetime fitness and phenotypic traits are taken under conditions that closely match those of the routine culture of the LHM base population.

For the purpose of assaying the flies for lifetime fitness and other traits such as sperm displacement, we also have backcrossed genetic markers [brown eye (bw), brown-eye-dominant (bwD), and nubbin wings (nubD)] and a compound-X \( [C(1)DXy] \) into the LHM base population. Each marker or chromosome has been backcrossed a minimum of 10 times through the LHM base population. Only the brow-eyed (LHM bw) and compound-X...
Reznick and is also illustrated by the transplantation experiments. Because the LHM population has such a long history of shown to rapidly adapt to new environments under field conditions. The capacity for rapid evolution to a new environment is manifest in newly derived Drosophila populations (for example, see ref. 44) and is also illustrated by the transplantation experiments of Reznick et al. (45) in which natural populations of guppies were shown to rapidly adapt to new environments under field conditions. Because the LHM population has such a long history of adaptation to a prescribed laboratory environment, we are able to measure both its genetic and fitness variation in the environment to which it is adapted, and thereby assess antagonistic coevolution within and between the sexes. Because the model organism is D. melanogaster, we can apply the powerful set of genetic tools available only in this species to take full advantage of the laboratory island population.

Stage 2: Cytogenetic Cloning and Construction of Hemiclones

There are many quantitative genetic techniques available to test for additive genetic variation for a trait and to measure net-selection on this variation. One of the most powerful techniques available with the D. melanogaster model system is the isolation and amplification of individual chromosomes through the use of balancers (which suppress recombination between homologous chromosomes). With this technique, many different chromosomes can be randomly sampled, and genetic variation can then be measured among different isochromosomal lines. Because the individual chromosomes can be amplified to many copies, high statistical power can be achieved.

A limitation with the use of balancers to construct isochromosome lines is that balancers are effective in suppressing recombination only when used on single chromosomes. Therefore the entire genome cannot be screened simultaneously with this technique. To eliminate this problem, we (in collaboration with my former postdoctoral associate, A. Chippindale, and former graduate student J. Gibson) have devised a method that relies on the lack of homologous recombination in male Drosophila, rather than balancers, to clonally amplify chromosomes, as originally described in ref. 14. The advantage of this technique is that a haplotype spanning nearly the entire genome (99.5%, including the X and both major autosomes, but excluding the dot fourth chromosome) can be clonally amplified simultaneously. As a consequence, nearly the entire genome of D. melanogaster can be screened for genetic variation (and selection, see below) simultaneously.

The protocol for cytogenetic cloning has been described in detail (43), and it is schematically outlined in Fig. 3. A random male is drawn from the base population and mated to a “clone-generator” female. These females carry a random Y chromosome from the LHM base population, an attached X (both X chromosomes in females cosegregate as a single linkage unit), and a translocation of the two major autosomes (which, in heterozygotes, causes the two autosomal chromosomes to cosegregate as a single linkage unit, among the living offspring). A single son from this cross (carrying a random genomic haplotype from the base population, which includes the X and the two major autosomes) is randomly selected and mated to many clone-generator females. The sons from these crosses that are retained (half of all sons are retained; the discarded half are homozygous for the translocation and are identifiable by the recessive markers on the translocation) carry the same genomic haplotype as their father and are next crossed to many clone-generator females to produce a clonal amplification line that perpetuates the genomic haplotype (Fig. 3).

Males from a clonal amplification line are next crossed to one of two types of females: wild-type females from the LHM base population or females from an attached-X replica of the LHM base population that is continuously backcrossed to the LHM population (Fig. 3). Half of the males or females from these crosses are completely wild type (the other half express a dominant genetic marker, bwD, and are discarded) and constitute a hemicleone. Members of a hemicleone share in common one nearly complete genomic haplotype, each expressed in a different random genetic background. A hemicleone is equivalent to the offspring that would be produced by randomly picking a group of eggs from the base population and then fertilizing each egg with a cloned copy of the same sperm.

Stage 3: Measuring Genetic Variation

To measure genetic variation for an arbitrary trait in the base population, multiple genomic haplotypes are independently sampled, cytogenetically cloned, and used to construct clonal amplification lines (Fig. 3). Next, hemicleones are constructed independently two or more times from each clonal amplification line, and the phenotypic value of each individual in each hemicleone is measured. Finally, random-effects analysis of variance is used to partition phenotypic variation among and between hemicleones to estimate additive genetic variance and heritability (43). Individuals within a hemicleone share half of their genetic variation in common, so that two times the additive genetic variation among hemicleones divided by the total phenotypic variation approximately estimates the heritability of the trait in the base population.
The additive genetic variation among hemiclones contains no nonadditive dominance variation, nor epistatic variation between alleles that reside in the genomic haplotype of a hemiclone and those in its genetic background. It does, however, potentially contain nonadditive epistatic variation between nonallelic genes that reside in the same genomic haplotype. Epistasis can occur between nonallelic genes that reside in genomic haplotypes inherited from (i) the father, (ii) the mother, or (iii) a mixture of these two. Only epistasis between genes that both reside in the paternal haplotype are included in the measure of additive genetic variation among hemiclones (a quarter of the four possible pair-wise types). The inclusion of some epistatic variation in the estimate of additive genetic variation is not unique to hemiclonal analysis. In fact, because of the lack of recombination in male *Drosophila*, it is a confound that is shared in common with most forms of quantitative genetic analysis with *Drosophila*. For example, a paternal half-sib design to estimate additive genetic variance includes epistatic variance among alleles that reside on the same chromosome because lack of recombination in male *Drosophila* keeps these alleles together during meiosis. Similarly, the well known North Carolina II breeding design (46) also confounds additive and epistatic variation, when applied to *D. melanogaster*, because the protocol uses balancer chromosomes that cause whole chromosomes to segregate like single giant supergenes (e.g., see ref. 47).

In effect the hemiclonal analysis technique of estimating additive genetic variation among genomic haplotypes treats the genome as if it were a single highly pleiotropic locus in males (but not in females). In the more conventional procedures of paternal half-sib analysis of variance and offspring-sire regression in *D. melanogaster*, the male genome segregates as if it were three gene loci (corresponding to the X and the two major autosomes, and ignoring the 0.5% of the genome found on the dot fourth chromosome and the small number if genes residing on the Y sex chromosome). Recombination occurs in females in paternal half-sib analysis of variance and offspring-sire regression designs (as it does in hemiclonal analysis), but these recombined chromosomes do not contribute to the covariance used to estimate heritability. The lack of recombination in males causes each male chromosome to be transmitted intact from father to offspring; hence, each pair of homologous chromosomes in males behaves like a pair of alleles residing at a single highly pleiotropic locus. The only way that we see to disentangle epistatic variation from estimates of additive genetic variance in *D. melanogaster* that are free from confounding maternal effects would be to carry out analysis of more distant paternal relatives. Because our measures of additive genetic variation among hemiclones include a limited amount (25%) of the potential epistatic variation, they represent an upper bound for the level of additive genetic variation among diploid individuals.

**Stage 4: Measuring the Net Selection Gradient on Standing Phenotypic Variation**

To measure the net selection gradient on phenotypic variation, we needed to first measure the average net-fitness associated with each hemiclone. To estimate this value, a prescribed number of eggs from a hemiclone are placed in a vial with competitors (we have specifically used a 1:2 ratio of hemiclonal eggs to competitor eggs in previous assays; see ref. 43). Next, the vials of eggs are put through the 2-day adult competition phase of their 2-week generation cycle. The treatments were (i) a “male-protected” environment in which the 10 hemiclonal females competed with 6 unrelated males for the resource that limited their lifetime fecundity (10 mg of live yeast, applied to the surface of the killed-yeast medium) in the absence of persistent courtship from males (no males present), and (ii) a “male-exposed” environment that was identical to the former treatment except that females competed in the presence of males at a 1:1 sex ratio of males to female, i.e., they competed under the social environment experienced during the normal propagation of the LH_M base population. The environmental conditions under which the flies were assayed (for example, timing of events, food levels, and densities and ages of flies) closely matched those to which the LH_M base population had adapted for >300 generations. Finally, the lifetime fecundity of the females was compared between the two treatments by measuring egg production during the last 18 h of their 2-week generation cycle (which is equivalent to egg production in the oviposition vials during the normal propagation of the LH_M base population). Any reduction in fecundity in the male-exposed compared with the male-protected treatments estimated the total cost of interacting with males, that is, the reduction in fecundity owing to resources spent by females when they defended, kicked, and otherwise responded to persistent interactions with males. Control experiments demonstrated that males did not compete with females for their limiting resource (live yeast) and that differences in fly density did not contribute to the difference in fecundity between treatments (42).

All 35 hemiclones experienced a decline in lifetime fecundity due to their interactions with males (Fig. 4, modified from ref. 42). On average, lifetime fecundity was reduced by 15.4% in the male-exposed treatment relative to the male-protected treatment. However, some hemiclones were harmed more than others (Fig. 4), and this heterogeneity in the degree to which lifetime fecundity was reduced by interactions with males measured variation for female resistance to male-induced harm. Analysis of variance was used to test for and estimate heritable variation among hemiclones for the degree of resistance to males, and highly significant genetic variation was observed (*P* < 0.0001; ref. 42). Heritable variation among hemiclones was estimated to contribute only 2.4% of the total phenotypic variation among individuals, indicating that standing heritable variation was low, as would be expected for a polygenic trait subject to strong directional selection. However, 17% of the total genetic variation among hemiclones for lifetime fecundity was due to variation in female resistance to male-induced harm, indicating that this trait contributed substantially to total genetic fitness variation among females.

Because males harm females through their seminal fluid (28), the rate of secondary mating with different males (i.e., remating rate) was a candidate phenotype contributing to female resis-
tance. We measured this trait as the percentage of females that remated at least once during their 2 days in the adult competition vials. We found significant heritable variation among hemiclones for remating rate (heritability = 18.2%, additive coefficient of variation = 14.4%, P < 0.0001, Fig. 5, modified from ref. 42). Female hemiclones that remated at higher average rate experienced a higher proportional reduction in their lifetime fecundity (P < 0.0001), indicating that reluctance to remate contributed substantially to the female resistance phenotype (42). We also found a significant negative correlation between our measure of female remating rate and female lifetime fecundity (P = 0.0269; Fig. 5), indicating that there was a negative selection gradient on female propensity to remate. Because (i) there was virtually no adult mortality during the 2-week generation cycle of the LH_M base population (43), (ii) there is no measurable correlation between adult and juvenile fitness (43), (iii) this negative correlation indicated a negative net-selection gradient on female remating rate. At this time, we have not completed an independent assay of lifetime fitness for the 35 hemiclones, so we were unable to directly test for a net-fitness selection gradient on female remating rate. However, an independent assay of a separate, smaller set of 16 hemiclones for which we have completed both a total fitness assay (48) and an assay for remating rate (T.A.L., E.H.M., and W.R.R., unpublished results) corroborated a negative net selection gradient on remating rate.

To test for inter-locus antagonistic coevolution between the sexes, the same 35 hemiclones were expressed in males and assayed for remating rate. The logic underlying this second assay was to determine whether there was additive genetic variation for remating rate in males, whether this variation was at least partially nonoverlapping with that controlling remating in females, and whether the net-selection gradient on remating rate in males was positive.

The protocol for measuring remating rate in males followed that of the male-exposed treatment of the female resistance assay except that the hemiclones were expressed as males and the females expressed random genotypes drawn from the base population (LH_M-bw). We found significant additive variation among male hemiclones for remating rate (proportion of non-virgin females that remated at least once with the hemiclonal males during their 2 days in the adult competition vials (heritability = 1.4%, additive coefficient of variation = 8.7%, P < 0.05; Fig. 6 and ref. 49). We also found a significant positive correlation between male remating rate and male adult fitness measured during the assay (P = 0.0081, Fig. 6). Because we found (i) no negative correlations between male remating rate and any other adult male fitness components, and (ii) past work in our laboratory found no measurable correlation between adult and juvenile fitness (43), the positive correlation between male adult offspring production and male remating rate indicated a positive net-selection gradient on this character in males. Again, because we have not yet completed an independent assay of net fitness variation among the 35 male hemiclones, we were unable to directly test for a positive net-selection gradient on male remating rate. However, a smaller assay of 17 different hemiclones was available from a related study that tested for both remate rate (49) and lifetime fitness (48). Here, a positive correlation was found between male remating rate and male lifetime fitness (P = 0.029), corroborating the significant positive net-selection gradient on this trait deduced from the measures of male adult lifetime offspring production from the larger sample of 35 hemiclones.

In sum, we found significant additive genetic variation among hemiclones for remating rate in both males and females, but the net-selection gradient on this trait was positive in males and negative in females. To look for independent genetic variation for remating rate in males and females, we constructed a bivariate plot of remating rate of the same hemiclones when expressed in males vs. females. No significant correlation was found (r = −0.175, P = 0.316), indicating that remating rate in the two sexes is controlled by different genetic variation. Because
there is independent genetic variation for remating rate in the two sexes, and because it is selected in opposite directions in each sex, we conclude that this trait presently is evolving in opposite directions in the two sexes and therefore that sexually antagonistic coevolution for mating rate is currently in evidence in this laboratory island population.

**Interpretation of Results from Hemiclonal Analysis**

In the above section, we used hemiclonal analysis to provide evidence that (i) females have genetic variation for resistance to male-induced harm, (ii) resistance contributes substantially to total genetic variation for net fitness, (iii) propensity to remating strongly influences the degree of female resistance, and (iv) there is unique genetic variation for remating rate in males and females that is selected and evolving in opposite directions in the two sexes. These data provide support for the hypothesis that perpetual inter-locus, intersexual arms races contribute to rapid genetic divergence among allopatric populations, and owing to the phenotypes that coevolve (reproductive behavior, physiology, and anatomy) are likely to be contributing to the specific genetic divergence that leads to reproductive isolation and speciation.

The data that we described, however, came from a laboratory island population rather than directly from nature. Some might argue that such populations are too artificial and hence tell us nothing about evolution in nature. We disagree. We cannot statistically extrapolate from our laboratory island population to natural populations of *D. melanogaster* because our laboratory population is not a random sample from the natural environment. We can, however, use laboratory island populations to make inferences about the fundamental principles of evolution and then use logic to extrapolate to the process of evolution in nature. Just as Darwin (50) used his study of island tortoise populations to deduce general evolutionary principles (rather than extrapolate to specific continental populations of tortoises), we used a laboratory island population to assess the evolutionary principles that underlie inter-locus antagonistic coevolution between the sexes. Our finding, that after hundreds of generations of coevolution we can detect an ongoing arms race between the sexes, supports the conclusion that perpetual arms races occur in nature and contribute substantially to the genetic divergence that leads to reproductive isolation and speciation.

A study such as ours could not feasibly be carried out in nature and therefore is possible only in the context of laboratory island populations. For example, the fact that we were able to detect heritabilities among hemichromes of only 2.4% for female resistance illustrates the substantial statistical power of this approach. The genetic measurements that we obtained for standing genetic variance and heritability took advantage of a broad array of genetic tools that are available only in laboratory populations of *D. melanogaster*. So, in general, we see two options: (i) study only natural populations and wait for technology to advance to the point that experiments such as ours are possible in situ, or (ii) study laboratory island populations where these experiments are possible today. We see a clear advantage to the second option.

We believe that the process of biotic evolution has basic underlying principles that apply to manmade microcosms just as they do to natural ecosystems. If we want to estimate the current or historical trajectory of a natural population, then we need to study that population in situ. But, if we want to understand the evolutionary principles that underlie evolution in nature, rather than specific evolutionary histories, then we can study them just as effectively, and in general more so because of reduced technical constraint, in the context of laboratory island populations. There is the danger that newly constructed laboratory populations will display misleading transients, and, as a result, caution is needed when interpreting results from laboratory populations that have not coevolved over a protracted number of generations (51). Nonetheless, laboratory island populations make possible evolutionary analysis that cannot be achieved in nature and thereby provide an essential complement to direct studies of populations in nature.

**Conclusions**

The allopatric model of speciation, as originally articulated by Dobzhansky (2) and Mayr (1), requires genetic divergence among physically isolated populations. Although sequence data are available for only a small number of genes that cause reproductive isolation, the available data indicate that these genes evolve rapidly under positive Darwinian selection [see article by H. A. Orr (52) in this supplement]. A fundamental question that remains is the identification of the selective process that drives the rapid divergence of the genes that lead to speciation. In this article, we show that experimental evolution, and more specifically hemiclonal analysis, provides support for the hypothesis that inter-locus antagonistic coevolution promotes rapid genetic divergence among allopatric populations.

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