

# Cytonuclear Disequilibrium in *Chrysochus* Hybrids Is Not Due to Patterns of Mate Choice

KIRSTEN J. MONSEN, BARBARA M. HONCHAK, STEFANIE E. LOCKE, AND MERRILL A. PETERSON

From the Department of Biology, Western Washington University, 516 High Street Bellingham, WA 98225-9160 (Monsen, Honchak, Locke, and Peterson). Kirsten J. Monsen is now at the Department of Biology and Molecular Biology, Montclair State University, 1 Normal Avenue, Montclair, NJ 07043; Barbara M. Honchak is now at the Department of Biological Sciences, Northern Arizona University, PO Box 5640, Flagstaff, AZ 86011-5640; and Stefanie E. Locke is now at the Department of Biology, West Chester University of Pennsylvania, West Chester, PA 19383.

Address correspondence to K. J. Monsen to the address above, or e-mail: monsenk@mail.montclair.edu.

---

## Abstract

We investigated patterns of cytonuclear disequilibrium between nuclear allozyme loci and partial mitochondrial COI and COII restriction fragment length polymorphism patterns within a population of hybridizing chrysomelid beetles and assessed to what degree the genotype frequencies of F1 hybrids were consistent with patterns of mate choice or endosymbiont infection. We document that in this population,  $\geq 50\%$  of the heterospecific pairs at a given time are composed of *Chrysochus auratus* females and *Chrysochus cobaltinus* males, suggesting that at least half of the F1 hybrids should possess the *C. auratus* mitochondrial genotype. However, we found that the majority (89%) of F1 hybrids possessed *C. cobaltinus* mtDNA ( $P < 0.001$ ). The lack of evidence for *Wolbachia* infection in these highly promiscuous beetles, coupled with the fact that F1 hybrids of both cross types do exist, indicates that endosymbionts are an unlikely explanation for the discrepancy between cytonuclear genotype frequencies and behavior. We argue that cytonuclear disequilibrium at this focal *Chrysochus* hybrid site is likely due to a strong directional bias in postmating prezygotic barriers in this system. The results presented here underscore the importance of combining both field and molecular data in studies of cytonuclear disequilibrium and point to the dangers inherent in attributing patterns of cytonuclear disequilibrium to assortative mating.

---

Patterns of cytonuclear disequilibrium can facilitate the investigation of a diverse array of evolutionary forces and biological processes in hybridizing taxa (Asmussen et al. 1987; Arnold 1993; Asmussen and Basten 1994). Specifically, studies of cytonuclear disequilibrium (the nonrandom association between nuclear and cytoplasmic genotypes) have examined levels of gene flow (e.g., Sites et al. 1996; Dessauer et al. 2000; Latta et al. 2001), directionality of hybridization (Lamb and Avise 1986; Sites et al. 1996; Harrison and Bogdanowicz 1997; Perry et al. 2001), and the mechanisms of selection on hybrids (Forbes and Allendorf 1991; Castro et al. 1999; Dessauer et al. 2000; Perry et al. 2001). Additionally, studies of cytonuclear disequilibrium have been used to elucidate how hybrid zones have formed (e.g., Harrison and Bogdanowicz 1997), and it has been suggested that measures of cytonuclear disequilibrium can be used to determine the age of reproductive barriers between species (Fu and Arnold 1991; Arnold 1993). Furthermore, it is possible to gain insight into intrinsic barriers to reproduction if patterns of cytonu-

clear disequilibrium are discordant with predictions based on observations of mating behavior or natural history (e.g., phenology of mating).

Cytonuclear disequilibrium is expected to arise in hybrid populations when there is nonrandom mating among the hybridizing taxa, high levels of migration of individuals with different cytonuclear genotypes from nearby populations, epistatic interactions between the nuclear and cytoplasmic genomes affecting hybrid fitness, or a postmating barrier to reproduction (Asmussen et al. 1987). Nonrandom mating may be due to assortative mating or differences in relative abundance of species and/or sex over space or time. Additionally, bacterial endosymbionts (e.g., *Wolbachia*, *Cardinium*) are known to cause a variety of reproductive anomalies (Werren 1997; Hunter et al. 2003) including skewed sex ratio (Fialho and Stevens 2000; Dyer and Jaenike 2005) that may lead to cytonuclear disequilibrium. Fertility and cytoplasmic compatibility can be affected by these infections if one animal is infected and the other is not or if the animals are

infected with different strains of bacteria. Although many studies have used cytonuclear patterns to elucidate the fitness of hybrids (Bernatchez et al. 1995; Wilson and Bernatchez 1998; Cianchi et al. 2003; Diniz et al. 2003; Won et al. 2003) and the occurrence of nonrandom mating (Avise and Saunders 1984; Lamb and Avise 1986; Malmos et al. 2001), to our knowledge, few, if any, studies have attempted to test predicted patterns of cytonuclear disequilibrium based on known mating pair composition in a natural population (but see Veen et al. 2001). For example, the classic study of Lamb and Avise (1986) showed that patterns of cytonuclear disequilibrium in a frog (*Hyla*) hybrid zone were consistent with predictions based on the stereotypical calling locations of the males of each species. They thus argued that the cytonuclear disequilibrium in this hybrid zone was due to mating behaviors, but did not directly quantify mate choices to confirm this explanation. However, because multiple factors might produce a pattern of cytonuclear disequilibrium, it is dangerous to infer a specific cause without direct support. For example, in the case of the *Hyla* hybrid zone, the cytonuclear disequilibrium might have been due to postmating prezygotic barriers (e.g., conspecific gamete preference, Howard 1999), rather than mating behaviors. Here, we document the hazards of inferring patterns of mate choice from measures of cytonuclear disequilibrium, by demonstrating a discordance between patterns of mate choice and the cytonuclear genotypes of F1 hybrids in a beetle hybrid zone.

Recent work in the hybrid zone involving leaf beetles in the genus *Chrysochus* has demonstrated that the 2 species, *Chrysochus auratus* and *Chrysochus cobaltinus*, mate assortatively in the laboratory and field (Peterson, Honchak, et al. 2005). Furthermore, this same study documented that at a focal hybrid zone site, most hybridizing pairs are composed of *C. auratus* females and *C. cobaltinus* males. In this study, we assess the generality of this pattern and test the predicted pattern of cytonuclear disequilibrium expected based on field observations of mating pair composition. In addition, we assess whether cytonuclear disequilibrium might be explained by *Wolbachia* infection in either or both species. Subsequently, we test our prediction of mitochondrial and nuclear genotype associations by measuring cytoplasmic disequilibrium within the same focal site.

### The *Chrysochus* Hybrid Zone

*Chrysochus auratus* and *C. cobaltinus* are the only North American representatives of the chrysomelid genus (Arnett 1968; Dobler and Farrell 1999). For most of their geographic ranges, these sister species are allopatric, with *C. auratus* present in eastern North America and *C. cobaltinus* restricted to western North America (Arnett 1968; Dobler and Farrell 1999; Peterson et al. 2001). The 2 species are sympatric in south-central Washington State where they form a 75-km-wide hybrid zone (Peterson et al. 2001; Peterson, Monsen, et al. 2005). Dobler and Farrell (1999) have shown a 6.5% sequence divergence in mtDNA, and species-specific allozyme alleles provide further evidence of the degree of divergence between the 2 species (Peterson et al. 2001). High divergence at both mitochondrial and nuclear loci in addition to differ-

ences in diet breadth (Dobler and Farrell 1999) and morphology (Peterson et al. 2001) suggests that this hybrid zone has formed as the result of secondary contact. Although F1 hybrids often compose up to 15% of a given population, there is strong evidence that they successfully reproduce only rarely (Peterson, Monsen, et al. 2005), resulting in a bimodal hybrid zone (sensu Jiggins and Mallet 2000) with parentals and F1 hybrids but effectively no F2's or backcross progeny. Additionally, selection against hybridization may vary between the species and sexes, as suggested by a species-specific pattern of the reproductive character displacement of male mate preference (Peterson, Honchak, et al. 2005). Given that selection pressures may be variable among sex and species, determining if there are nonrandom associations between nuclear and mitochondrial markers will be useful in assessing where potential barriers to reproduction may have evolved or are expected to evolve. For example, the degree to which females are able to employ conspecific sperm precedence may influence the strength of selection against hybridization faced by those females (Marshall et al. 2002).

### *Chrysochus* Mating Behavior

Both *Chrysochus* species are highly promiscuous in natural populations, with adults of both sexes averaging approximately one mating per day (Dickinson 1995). Males of both species practice postcopulatory mate guarding often for times >1.5 h. Because of their promiscuous behavior combined with postcopulatory mate guarding, 28–36% of a population will be paired at any one time (Dickinson 1995), simplifying field observations of mating behavior. Although patterns of mate choice in the hybrid zone have been explored in both the laboratory and the field (Peterson, Honchak, et al. 2005; Peterson, Monsen, et al. 2005), mitochondrial markers have not been used in prior genetic analyses of F1 hybrids in this system (e.g., Peterson et al. 2001; Peterson, Monsen, et al. 2005). Thus, we have not previously assessed whether there is a directional bias in the parentage of F1 hybrids.

## Materials and Methods

### Mating Pair Composition

Sex and species composition of heterospecific mating pairs were documented within the focal site in the *Chrysochus* hybrid zone that has been described previously (Peterson, Honchak, et al. 2005; Peterson, Monsen, et al. 2005). Following methods described previously (Peterson, Honchak, et al. 2005), such surveys were performed on 4 separate dates (1 July 2000, 9 July 2000, 24 June 2003, and 22 July 2004), to determine if there is a directional bias in the composition of mating pairs at this site, and if so, whether that bias is temporally stable. The results of the 1 July 2000 survey have been reported elsewhere (Peterson, Honchak, et al. 2005). Compared with other nearby habitat patches, the focal site used for these surveys features unusually high population sizes of both parental species, with population sizes exceeding those in nearby patches by 1–3 orders of magnitude (Peterson,

Honchak, et al. 2005). These higher population sizes are due to the combined effects of a large host plant patch and high densities of beetles. Although similarly high population sizes occur in a few allopatric populations of both species in the region, all of the other hybrid zone sites have either small host plant patches, low beetle densities, or both (Peterson, Honchak, et al. 2005).

## Molecular Methods

We have previously shown species and hybrid identification based on morphological characteristics to be extremely accurate (Peterson, Monsen, et al. 2005). However, to verify species identification, we screened 92 putative *C. auratus* (42 collected in 1999 and 50 collected in 2000), 92 *C. cobaltinus* (42 collected in 1999 and 50 collected in 2000), and 141 putative F1 hybrids (collected in 2000) from the focal site with 3 species-specific allozyme loci (Peterson et al. 2001) and species-specific mtDNA restriction fragment length polymorphism patterns (Dobler and Farrell 1999) to verify species identification and to test for cytonuclear disequilibrium in F1 hybrids.

Individuals used in the allozyme analysis were screened for species-specific alleles for *AAT*, *IDH*, and *MDH* following the methods of Peterson et al. (2001). Additionally, total genomic DNA was extracted from a single leg of each individual used in the allozyme analysis using the QiaAmp Mini DNA Kit (Qiagen, Valencia, CA). A 1154-bp fragment of mtDNA was amplified from total genomic DNA using primers C2-N-3661 (Simon et al. 1994) and MP1 (5' ATTAAGTTTTTGTGACTAGCA3') based on the partial sequence of *C. cobaltinus* COI and COII genes. Polymerase chain reaction (PCR) amplifications were carried out in 2 separate 50  $\mu$ l reactions using the following components and conditions:  $\sim$ 100 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl pH 9, 0.1% Triton X-100, 1.5 mM MgCl<sub>2</sub>, 0.8  $\mu$ M of both the forward and reverse primers, 0.2 mM dNTPs, 5 units *Taq* polymerase, and water to a final volume of 50  $\mu$ l. Amplification was carried out in a Perkin-Elmer 9600 thermocycler under the following conditions: 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, 46°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 7 min. Both 50  $\mu$ l reactions were pooled for each individual to produce 100  $\mu$ l of PCR product. It was necessary to have two 50- $\mu$ l reactions because 100  $\mu$ l was too large a reaction volume to run efficiently in the Perkin-Elmer 9600 thermocycler. PCR fragments were purified using the QiaQuick PCR purification kit (Qiagen). Purified mtDNA fragments were digested under standard conditions with 3 enzymes, *AluI*, *DraI*, and *HinfI*, that collectively included 11 informative, species-specific restriction sites (Dobler and Farrell 1999). Digests were run on 2% 1 $\times$  Tris, acetic acid, EDTA buffer (TAE) gels and stained with Sybr Gold (Molecular Probes, Eugene, OR).

## Wolbachia Screen

Reproductive tissues were dissected from 2 males and 2 females of both species from the focal site. Other recent studies that have detected the presence of *Wolbachia* infection

have screened a similar number of individuals (Clark et al. 2005; Haine and Cook 2005). Additionally, given the promiscuous mating behavior of both *Chrysochus* species, it is highly likely that if *Wolbachia* were present, it would be present in almost all individuals in a population. Additionally, because both sexes of both species form heterospecific pairs, it is likely that if one species were infected with *Wolbachia*, the other species would become infected as well. DNA was extracted from the reproductive tissues using the QiaAmp Mini DNA Kit (Qiagen). The bacterial cell-cycle gene *ftsZ* was amplified using the primers *ftsZF1* and *ftsZR1* (Werren et al. 1995) in 25  $\mu$ l reactions under the same conditions as described above for mtDNA amplification using a 45°C annealing temperature. Additionally, the polymerase used for the *Wolbachia* screen was a combination of 2 polymerases specifically designed for long PCR (Expand enzyme system, Boehringer Mannheim, Indianapolis, IN). Long PCR is more sensitive to the presence of *Wolbachia* DNA among insect genomic DNA than standard PCR (Jeyaprasanth and Hoy 2000). All *Wolbachia* screens were run with a positive control of *Nasonia pulex*, known to be infected with *Wolbachia*. Positive control DNA was extracted from the entire *N. pulex* animal and not the isolated reproductive tissues.

## Statistical Analyses

To assess whether the composition of heterospecific pairs at the focal site was asymmetric, we analyzed data from each date separately, using binomial tests of the hypothesis that the 2 directions of heterospecific crosses were equally likely. Cytonuclear disequilibrium was assessed by examining whether each mtDNA haplotype was represented in 50% of F1 hybrids, again using a binomial test (SPSS 2003). We did not use existing software that is specifically designed to assess cytonuclear disequilibrium (Asmussen and Basten 1994), because that software is constrained to using a random sample of parentals and hybrids, taken at the frequency with which they occur in nature. We specifically over-sampled F1 hybrids at this location compared with their frequency because these individuals are most informative for assessing the concordance between patterns of mate choice by parentals and F1 cytonuclear genotype frequencies.

## Results

All 92 *C. cobaltinus* and all 92 *C. auratus* genotyped with species-specific nuclear and mitochondrial markers showed the expected species-specific patterns. Additionally, all putative 141 F1 hybrids screened were heterozygous at all 3 nuclear allozyme loci. Because our phenotype-based identifications were 100% accurate, we are confident in our identifications of the identities of mating individuals observed in the field. During all observations at the focal hybrid zone site, the composition of heterospecific pairs was either statistically indistinguishable from symmetric or featured a biased over-representation of female *C. auratus* paired with a male *C. cobaltinus*, compared with the opposite cross (Table 1). On 1 July 2000, 64.1% of the 39 heterospecific pairs ( $P = 0.078$ )

**Table 1.** Binomial test of asymmetry in heterospecific pair composition at the focal site

Date	No. of heterospecific pairs observed	AF × CM	CF × AM	P value
1 July 2000	39	25 (0.641)	14 (0.369)	0.078
9 July 2000	81	50 (0.617)	31 (0.393)	0.034
24 June 2003	29	23 (0.793)	6 (0.217)	0.002
22 July 2004	4	2 (0.50)	2 (0.50)	1.0

AF × CM represents the frequency of heterospecific pairs composed of *Chrysobothris auratus* females and *Chrysobothris cobaltinus* males. CF × AM represents the frequency of heterospecific pairs composed of *Chrysobothris cobaltinus* females and *Chrysobothris auratus* males.

featured a *C. auratus* female and *C. cobaltinus* male (reported previously in Peterson, Honchak, et al. 2005, without a statistical test of directional bias). This combination represented 61.7% of 81 heterospecific pairs observed on 9 July 2000 ( $P = 0.034$ ) and 79.3% of 29 such pairs on 24 June 2003 ( $P = 0.002$ ) at the focal hybrid zone site. On 22 July 2004, only 4 heterospecific pairs were encountered, of which 2 featured a *C. auratus* female paired with a *C. cobaltinus* male ( $P = 1.0$ ). Thus, in general, the composition of heterospecific pairs would conservatively suggest that at least 50% of F1 hybrids should have *C. auratus* mothers.

There was a significant pattern of cytonuclear disequilibrium in F1 hybrids, such that 11% of F1 hybrids featured the *C. auratus* mtDNA haplotype, whereas 89% had the *C. cobaltinus* haplotype, a pattern discordant with expectations based on random mating ( $P < 0.001$ ). Given that one would expect at least 50% of F1 hybrids to have the *C. auratus* mtDNA haplotype, the binomial test is a conservative test for the pattern of cytonuclear disequilibrium based on actual patterns of mate choice. The skewed cytonuclear genotypes of F1 hybrids were apparently not attributable to patterns of *Wolbachia* infection. The positive *N. pulex* control produced the expected ~1054 bp *ftsZ* fragment, demonstrating that long PCR was sensitive enough to amplify *Wolbachia* DNA in the presence of insect genomic DNA. This positive control was conservative because the DNA was extracted from the entire *N. pulex* animal, not just the reproductive tissue. However, this fragment did not amplify in any of the *Chrysobothris*, indicating that there is no apparent *Wolbachia* infection in either species at the focal site.

## Discussion

Through direct quantification of patterns of cytonuclear disequilibrium and mate choice, our study illustrates the dangers inherent in indirectly drawing inferences about mating behaviors from measurements of cytonuclear disequilibrium. Specifically, although the composition of heterospecific pairs in the focal *Chrysobothris* population suggested that most F1 hybrids should have the *C. auratus* mtDNA haplotype, most hybrids actually had the *C. cobaltinus* haplotype. The relative abundance of the 2 species at the focal hybrid zone site is temporally stable (including 1999, the generation that pro-

duced the hybrids screened in the present study; Peterson, Honchak, et al. 2005). In addition, there is no evidence of dramatic swings in the proportional composition of heterospecific pairs at this site. Therefore, it is unlikely that temporal variation in the composition of heterospecific pairs, resulting from differences in the relative abundance of each sex and species, explains the pattern of cytonuclear disequilibrium observed here. Additionally, we have genotyped 17 F1 hybrids collected from the focal site in 1999, of which 95% possessed the *C. cobaltinus* mtDNA haplotype (Peterson M, Tantalo L, Monsen K, Locke S, unpublished data), suggesting that the pattern of cytonuclear disequilibrium documented herein is robust.

The discordance between mating behaviors and F1 hybrid cytonuclear genotypes is apparently not due to patterns of endosymbiont infection (e.g., Werren 1997; Hunter et al. 2003). First, we found no evidence of *Wolbachia* infection in either *Chrysobothris* species. Second, endosymbiont-induced cytoplasmic incompatibility is not consistent with the observation that virgin females of both species of these highly promiscuous beetles produce viable F1 offspring when mated with heterospecific males (Peterson M, Monsen K, Larson E, Brassil M, Buckingham K, unpublished data). Third, male-killing endosymbionts (Fialho and Stevens 2000; Dyer and Jaenike 2005) are unlikely, given that the sex ratio of F1 hybrids at the focal site is consistently 50:50 (Peterson M, Monsen K, unpublished data).

In addition, it is highly unlikely that the excess of F1 hybrids with *C. cobaltinus* mtDNA haplotypes is due to immigration of F1 hybrids from nearby populations in which most heterospecific pairs feature *C. cobaltinus* females. The population size at the focal hybrid zone site is 1–3 orders of magnitude larger than at other nearby sites, and the relative abundance of the 2 parental species is similar in those nearby sites (Peterson, Honchak, et al. 2005). Furthermore, surveys conducted in those sites in 2000 and 2004 revealed a total of only 29 heterospecific pairs, of which 15 (51.7%) featured a *C. cobaltinus* female (Peterson M, Monsen K, unpublished data). Thus, based on the similar patterns of mate choice in the surrounding sites, compared with our focal site, we would expect a similar pattern of cytonuclear disequilibrium in populations surrounding the focal site. Additionally, dispersal capabilities in both species appear to be limited (Williams 1992; Dickinson 1995; St Pierre et al. 2005; Peterson M, Tantalo L, Holland J, unpublished data), suggesting that immigrants would not be sufficiently numerous to alter haplotype frequencies in F1 hybrids in the focal site.

Strong selection against F1 hybrids with *C. auratus* mitochondrial DNA and *C. cobaltinus* nuclear DNA could explain the pattern of cytonuclear disequilibrium observed in this system if offspring of this cross frequently fail to develop. Virgin *C. auratus* females mated only to *C. cobaltinus* males produce just as many first instar F1 hybrid larvae as do virgin *C. cobaltinus* females mated to only *C. auratus* males in laboratory studies (Peterson M, Monsen K, Larson E, Brassil M, Buckingham K, unpublished data). Thus, it is highly unlikely that differential mortality during early development

explains the observed pattern of cytonuclear disequilibrium, although it remains possible that differences in mortality during subsequent larval instars could underlie the pattern.

We argue that the most likely explanation for the cause of cytonuclear disequilibrium in F1 *Chrysochus* hybrids is that asymmetric postmating prezygotic barriers (e.g., conspecific sperm precedence, Howard 1999) exist in this system. Indeed, it has been suggested that bimodal hybrid zones (such as the *Chrysochus* hybrid zone) often feature assortative mating and/or assortative fertilization (Jiggins and Mallet 2000). Additionally, some taxa that appear to mate randomly are known to have assortative fertilization, suggesting that gamete recognition may evolve faster than mate recognition (Jiggins and Mallet 2000 and references within). Finally, postmating prezygotic barriers have proved to be asymmetric in many taxa (Howard 1999).

There is ample opportunity for conspecific sperm precedence in this system. Although males engage in postcopulatory mate guarding, females mate with multiple males (Dickinson 1995). These females, which can store sperm for extended periods (Peterson M, Monsen K, Larson E, Brassil M, Buckingham K, unpublished data), derive substantial fecundity benefits from mating with multiple males (Schwartz and Peterson 2006). Given our evidence against the other possible causes of cytonuclear disequilibrium in *Chrysochus* hybrids, asymmetric postmating prezygotic barriers appear to be the most plausible explanation for the strong bias in F1 hybrid mtDNA haplotypes, although it is premature to rule out asymmetries in hybrid viability during larval and/or pupal development. Clearly, future studies of the *Chrysochus* hybrid zone should focus on explicit tests of the hypothesis that such barriers exist in this system, and that they explain the pattern of cytonuclear disequilibrium documented in this paper.

Although we cannot yet definitively establish a cause for the biased mtDNA haplotype frequencies in *Chrysochus* hybrids documented in this paper, our results make the important point that one should not use patterns of cytonuclear disequilibrium as the sole basis for inferences regarding reproductive barriers between species. Unfortunately, such inferences are common in the literature (e.g., Avise and Saunders 1984; Lamb and Avise 1986; Avise et al. 1997; Mallet et al. 1998; Malmos et al. 2001). However, if we are to understand the cause of patterns of cytonuclear disequilibrium in hybrid zones, it is critical that we directly and rigorously examine each of the many factors that may produce such a pattern.

## Funding

Project Development Award from Western Washington University; Biology Department Research Funds; and the National Science Foundation (DEB-0212652).

## Acknowledgments

We thank M. Brassil, K. Buckingham, C. Fabry, J. Green, J. Holland, J. Mendoza, and L. Tantalo for their help in the field. In addition, we are grateful to B. Emerson, C. Yoon, and anonymous reviewers for insightful comments that improved an earlier version of this manuscript.

## References

- Arnett RH. 1968. The beetles of the United States (a manual for identification). Ann Arbor (MI): The American Entomological Institute.
- Arnold A. 1993. Cytonuclear disequilibria in hybrid zones. *Annu Rev Ecol Syst.* 24:521–554.
- Asmussen M, Arnold J, Avise J. 1987. Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. *Genetics.* 155:755–768.
- Asmussen M, Basten C. 1994. Sampling theory for cytonuclear disequilibria. *Genetics.* 138:1351–1363.
- Avise J, Pierce P, Vanden Avyle M, Smith M, Nelson W, Asmussen M. 1997. Cytonuclear introgressive swamping and species turnover of bass after an introduction. *J Hered.* 88:14–20.
- Avise J, Saunders N. 1984. Hybridization and introgression among species of sunfish. (*Lepomis*): analysis of mitochondrial DNA and allozyme markers. *Genetics.* 108:237–255.
- Bernatchez L, Glemet H, Wilson C, Danzmann R. 1995. Introgression and fixation of arctic char (*Salvelinus fontinalis*). *Can J Fish Aquat Sci.* 52: 179–185.
- Castro JA, Ramon M, Picornell A, Moya A. 1999. The genetic structure of *Drosophila subobscura* populations from the islands of Majorca and Minorca (Balearic Islands, Spain) based on allozymes and mitochondrial DNA. *Heredity.* 83:271–279.
- Cianchi R, Ungaro A, Marini M, Bullini L. 2003. Differential patterns of hybridization and introgression between swallowtails *Papilio machaon* and *P. hospiton* from Sardinia and Corsica islands (Lepidoptera, Papilionidae). *Mol Ecol.* 12:1461–1471.
- Clark ME, Anderson CL, Cande J, Karr TL. 2005. Widespread prevalence of *Wolbachia* in laboratory stocks and the implications for *Drosophila* research. *Genetics.* 170:1667–1675.
- Dessauer HC, Cole CJ, Townsend CR. 2000. Hybridization among western whiptail lizards (*Cnemidophorus tigris*) in southwestern New Mexico: population genetics, morphology, and ecology in three contact zones. *Bull Am Mus Nat Hist.* 246:4–148.
- Dickinson JL. 1995. Trade-offs between postcopulatory riding and mate location in the blue milkweed beetle. *Behav Ecol.* 6:280–286.
- Diniz NM, Soares AEE, Seppard WS. 2003. Genetic structure of honeybee populations from southern Brazil and Uruguay. *Genet Mol Biol.* 26:47–52.
- Dobler S, Farrell BD. 1999. Host use evolution in *Chrysochus* milkweed beetles: evidence from behaviour, population genetics and phylogeny. *Mol Ecol.* 8:1297–1307.
- Dyer KA, Jaenike J. 2005. Evolutionary dynamics of a spatially structured host-parasite association: *Drosophila innubula* and male-killing *Wolbachia*. *Evolution.* 59:1518–1528.
- Fialho RF, Stevens L. 2000. Male-killing *Wolbachia* in a flour beetle. *Proc Biol Sci.* 267:1469–1474.
- Forbes SH, Allendorf FW. 1991. Associations between mitochondrial and nuclear genotypes in cutthroat trout hybrid swarms. *Evolution.* 45:1332–1349.
- Fu YX, Arnold J. 1991. On the association of restriction fragment length polymorphisms across species boundaries. *Proc Natl Acad Sci USA.* 88:3967–3971.
- Haine ER, Cook JM. 2005. Convergent incidences of *Wolbachia* infection in fig wasp communities from two continents. *Proc R Soc Lond B Biol Sci.* 272:421–429.
- Harrison R, Bogdanowicz S. 1997. Patterns of variation and linkage disequilibrium in a field cricket hybrid zone. *Evolution.* 51:493–505.
- Howard DJ. 1999. Conspecific sperm and pollen precedence and speciation. *Annu Rev Ecol Syst.* 30:109–132.

- Hunter MS, Perlman SJ, Kelly SE. 2003. A Bacteroidetes-group bacterial symbiont induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella*. *Proc R Soc Lond B Biol Sci.* 270:2185–2190.
- Jeyaprakash A, Hoy MA. 2000. Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. *Insect Mol Biol.* 9:393–405.
- Jiggins CD, Mallet J. 2000. Bimodal hybridization zones and speciation. *Trends Ecol Evol.* 15:250–255.
- Lamb T, Avise J. 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. *Proc Natl Acad Sci USA.* 83:2526–2530.
- Latta R, Linhart Y, Mitton J. 2001. Cytonuclear disequilibrium and genetic drift in a natural population of ponderosa pine. *Genetics.* 158:843–850.
- Mallet J, McMillan WO, Jiggins CD. 1998. Estimating the mating behavior of a pair of hybridizing *Heliconius* species in the wild. *Evolution.* 52:503–510.
- Malmos KB, Sullivan BK, Lamb T. 2001. Calling behavior and directional hybridization between two toads (*Bufo microscaphys* X *B. woodhousii*) in Arizona. *Evolution.* 55:626–630.
- Marshall JL, Arnold ML, Howard DJ. 2002. Reinforcement: the road not taken. *Trends Ecol Evol.* 17:558–563.
- Perry W, Feder J, Dwyer G, Lodge D. 2001. Hybrid zone dynamics and species replacement between *Orconectes* crayfishes in a Northern Wisconsin lake. *Evolution.* 55:1153–1166.
- Peterson MA, Dobler S, Holland J, Tantalo L, Locke S. 2001. Behavioral, molecular, and morphological evidence for a hybrid zone between *Chrysochus auratus* and *C. cobaltinus* (Coleoptera: Chrysomelidae). *Ann Entomol Soc Am.* 94:1–9.
- Peterson MA, Honchak B, Locke S, Beeman T, Mendoza J, Green J, Buckingham K, White MA, Monsen K. 2005. Relative abundance and the species-specific reinforcement of male mating preference in the *Chrysochus* (Coleoptera: Chrysomelidae) hybrid zone. *Evolution.* 59:2639–2655.
- Peterson MA, Monsen KJ, Pederson H, McFarland T, Bearden J. 2005. Direct and indirect analysis of the fitness of *Chrysochus* (Coleoptera: Chrysomelidae) hybrids. *Biol J Linn Soc Lond.* 84:273–286.
- Schwartz SK, Peterson MA. 2006. Strong material benefits and no longevity costs of multiple mating in an extremely polyandrous leaf beetle, *Chrysochus cobaltinus* (Coleoptera: Chrysomelidae). *Behav Ecol.* 17:1004–1010.
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am.* 87:651–702.
- Sites J, Basten C, Asmussen M. 1996. Cytonuclear genetic structure of a hybrid zone in lizards of the *Sclerophorus grammicus* complex (Sauria, Phrynosomatidae). *Mol Ecol.* 5:379–392.
- SPSS Inc. 2003. SPSS 11.0.2 for Mac OSX. Chicago (IL): SPSS Inc.
- St Pierre MJ, Hendrix SD, Lewis CK. 2005. Dispersal ability and host-plant characteristics influence spatial population structure of monophagous beetles. *Ecol Entomol.* 30:105–115.
- Veen T, Borge T, Griffith S, Sætre GP, Bures S, Gustafsson L, Sheldon B. 2001. Hybridization and adaptive mate choice in flycatchers. *Nature.* 411:45–50.
- Werren JH. 1997. Biology of *Wolbachia*. *Annu Rev Entomol.* 42:587–609.
- Werren JH, Windsor D, Guo LR. 1995. Distribution of *Wolbachia* among neotropical arthropods. *Proc R Soc Lond B Biol Sci.* 261:55–63.
- Williams CE. 1992. Movement of the dogbane beetle, *Chrysochus auratus* (Coleoptera: Chrysomelidae) in a patchy environment. *Banisteria.* 1:8–10.
- Wilson C, Bernatchez L. 1998. The ghost of hybrids past: fixation of arctic char (*Salvelinus alpinus*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). *Mol Ecol.* 7:127–132.
- Won Y, Hallam S, O'Mullans G, Vrijenhoek R. 2003. Cytonuclear disequilibrium in a hybrid zone involving deep-sea hydrothermal vent mussels of the genus *Bathymodiolus*. *Mol Ecol.* 12:3185–3190.

Received September 1, 2006

Accepted February 15, 2007

Corresponding Editor: Ron Woodruff