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Effects of Relative Abundance on Sexual Isolation and Hybridization Risk in a Naturally Occurring Hybrid Zone of *Chrysochus* Leaf Beetles

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Abstract

Reinforcement theory is a controversial mechanism by which speciation can occur through reduced hybrid fitness promoting the evolution of reproductive isolating mechanisms. Populations within a hybrid zone may be at differing relative abundances which may affect their risk of hybridization and strength of sexual isolation. The effect of relative abundance has been seldom examined in studies of reinforcement. *Chrysochus cobaltinus* and *C. auratus* leaf beetles from a zone of secondary contact in central Washington were run in various multi-choice mating trials to determine if lab results were indicative of those occurring in nature and whether relative abundance had an effect on risk of hybridization, pairwise sexual isolation (PSI) and overall isolation (I_{PSI}). Results from lab experiments were found to be representative of those in nature. Relative abundance had a significant effect on both the risk of hybridization. In addition, our results suggested that overall sexual isolation and that as a species became rarer, they also became choosier. This sets the stage for further research on hybridization risk and relative abundance to be taken into the field setting, and indicates that studies of reinforcement should consider not only relative abundance, but also the relationship between relative abundance and choosiness, to better understand the risk of hybridization.

Introduction

Speciation research is a main focus in evolutionary biology, in an effort to understand how evolution has shaped the diversity of the natural world. Mayr (1970) defined a species as a reproductively isolated group, an idea more commonly known as the Biological Species Concept. This definition has become widely accepted, and as such, much of the current research on speciation is focused on reproductive isolating mechanisms (Coyne, 1992). When examining zones of contact between two closely related species, most studies have tended to focus on reproductive isolating mechanisms that arise to keep the species from interbreeding.

One way to do this is to examine species that have been separated due to some natural event, and have now come into a zone of secondary contact (Harrison, 1993).

Within this zone of sympatry, members of the two sister species or host races may begin to attempt interbreeding; if it is possible for offspring to be produced from these matings, a hybrid zone may form (Mayr, 1970; Peterson et al., 2001). However, in many cases, hybrids exhibit a marked fitness reduction from parentals (Mayr, 1970) and following evolutionary theory, natural selection should favor the evolution of traits that limit interspecific matings which result in hybrid formation (Dobzhansky, 1940). This may occur through the formation of reproductive isolating mechanisms, some of which may act postzygotically, such as hybrid sterility, or some which may act prezygotically, such as sexual isolation.

Sexual isolation is defined as individuals avoiding matings with another species or race (Gilbert & Starmer, 1985; Rolán-Alvarez & Caballero, 2000). The visible result of this phenomenon is assortative mating, in which more conspecific and fewer heterospecific matings occur than expected through random chance. This serves to reduce introgression or gene flow through hybridization (Mayr, 1970; Ribi & Oertli, 2000) and pushes the two groups farther apart. If this process is strengthened by reduced hybrid fitness then it is called reinforcement, as is seen in a variety of hybrid zones (Nosil et al., 2003; Servedio & Noor, 2003).

Relative abundance can be an important factor in determining the strength of selection against hybridization throughout the hybrid zone. Distinct populations will likely not have equal numbers of each species, especially those that lie near parental populations (Howard, 1993). Being rare should put a species at greater risk of hybridization because it is less likely to encounter those of its own species (Howard, 1993). Furthermore, at either extreme of the hybrid zone, the common species will often

be adjoining its parental population and exposed to gene flow from naïve individuals that will counteract selection against hybridization, while the rare species will be farthest from its parental population and under greater selection pressure to avoid hybridization (Howard, 1993). Thus the rare species should become choosier over time, compared to the common species.

The leaf beetles *Chrysochus cobaltinus* and *C. auratus* are sister species that have a 75 km wide zone of secondary contact in the Yakima River Valley, and are easily distinguishable by their differing elytral color and antennal morphology (Peterson et al., 2001, in press). They co-occur on patches of dogbane (*Apocynum cannabinum*) their preferred food, where the entire life cycle takes place (Peterson et al., 2001). These beetles interbreed and produce hybrids with an intermediate morphology. Although these F_1 hybrids engage in copulatory behavior as often as parentals (Peterson et al., unpub. data), genetic data indicate that they are essentially sterile (Peterson et al., in press). Nosil et al. (2003) have described three conditions that should be met in a system which could show reinforcement: interbreeding which leads to gene flow, reduced hybrid fitness and selection against hybrids. Thus the *Chrysochus* hybrid zone provides ideal conditions to study reinforcement and sexual isolation at work.

In this study, we examine several questions addressing reinforcement and sexual isolation. First, we ascertained whether the results of mate choice experiments in the lab are representative of what is occurring in nature. If they are, then it validates research on sexual isolation done in the lab setting, as in other studies (McLain, 1985; Rolán-Alvarez et al., 1999). Additionally, we examined whether sexual isolation and hybridization risk vary among populations and whether they are influenced by relative abundance. In

comparing sexual isolation among hybrid zone populations, we controlled relative abundance. In contrast, to determine the effect of relative abundance, population was held constant.

Materials and Methods

<u>General Procedures</u>:

For all mating experiments in this study, we used *Chrysochus auratus* and *C. cobaltinus* adults, collected from the hybrid zone in eastern WA in July and August 2003. Beetles were returned to the lab in a cooler and separated by species and sex. Species was determined by elytra color and antennal segment morphology. Sex was determined by prying open their genital aperture and identifying either an aedeagus (penis) or an ovipositor. Subsequently, all beetles were kept in plastic boxes in an incubator with a day/night cycle of 22°C/16°C. Boxes were cleaned 2-3 times a week and dogbane replaced as needed. All mating experiments followed a multi-choice design, using metal cages measuring 30cm x 30 cm x 60cm, containing 2-3 stalks of dogbane. For each experiment, we haphazardly placed 100 beetles in a cage in an environmental room (28°C, simulating daytime temperatures in eastern WA). Within each species, a 50:50 sex ratio was maintained at all times, but the relative abundance of species varied, depending on the experiment (see below).

All experiments were started near 9:00 AM because *Chrysochus* mate more actively in the early part of the day. After a 1.5 hour initial acclimation period, we recorded the sex and species of individuals in each mating pair in each cage every 1.5 hours, taking 5 minutes per cage for these observations. We repeated these observations for 7.5 hours each day, for three consecutive days (total of 15 observation periods per

cage). At the end of each day, the cages were removed from the environmental room and stored in the lab to reduce activity until the following day's trials. Dead or feeble individuals were replaced before each day's trials.

In general, for each objective, we tested the hypothesis that the dependent variables differed across mating cages, using ANOVA with LSD tests for post-hoc pairwise comparisons. Proportional data were angular-transformed prior to analysis. Because individual beetles were used in multiple trials within an experiment, these comparisons suffer from statistical nonindependence. However, reuse of beetles would be expected to homogenize results in our experiment, making our comparisons conservative.

<u>Objective-Specific Methods</u>:

Objective 1: Are multi-choice experiments in the lab representative of nature?

To address this question, we performed a multi-choice experiment with beetles from the hybrid zone site that has been the focus of numerous field and lab studies of mate choice (Peterson et al. 2001, in press, unpub. data).

To best simulate the naturally-occurring densities in this population (Peterson et al. in press), we used a 70:30 ratio of *C. auratus* to *C. cobaltinus*. To compare our results with results from the field (Peterson et al. unpub. data), we compared patterns of sexual isolation between males and females of each species, using the pair sexual isolation (PSI) statistic of Rolán-Alvarez & Caballero (2000). Values of PSI < 1 indicate fewer pairs than expected from random mating, while PSI values > 1 indicates more pairs than expected from random mating (Rolán-Alvarez & Caballero, 2000). PSI values were calculated using a BASIC computer program (Rolán-Alvarez & Caballero, 2000). To

determine if representative estimates of PSI could be obtained from single cages of 100 beetles, we also compared sexual isolation across three replicates of 100 beetles (different beetles in each replicate) from this site at a 50:50 species ratio.

Objective 2: Does sexual isolation vary among populations in the hybrid zone?

To compare sexual isolation across populations, we performed multi-choice experiments using beetles from each of three populations:1) the focal population (S) used in Objective 1, 2) a second centrally-located population (AF), occurring 5 km NNW of Population S, and 3) a population (AR) located within the hybrid zone near its western edge (~25 km WSW of Population S) . Because relative abundance may influence measures of sexual isolation (Objective 3), we held relative abundance constant by keeping each species ratio at 50:50 to isolate only the results due to source population.

Objective 3: Does the risk of hybridization depend on relative abundance?

To determine the relationship between relative abundance and hybridization risk, we performed a series of multi-choice experiments using beetles from site S, but varying the relative abundance of the two species. In addition to the 50:50 ratio used in Objective 1, the ratios of *C. cobaltinus* : *C. auratus* were 90:10, 30:70, 70:30 and 10:90. The probability of heterospecific mating was determined for each species and sex.

Objective 4: Does relative abundance influence patterns of sexual isolation?

Using the sexual isolation statistics developed by Rolán-Alvarez and Caballero (2000), PSI and I_{PSI} were calculated for each of the cages at varying abundances (see Objective 3). I_{PSI} is an overall (not pairwise) measure of sexual isolation which ranges from -1 to +1, where values over 0 indicate the presence of sexual isolation between two species (Rolán-Alvarez & Caballero, 2000).

Objective 5: Does choosiness vary with relative abundance?

To examine how choosiness is affected by the rarity of a species, simulations of various situations corresponding to varying levels of choosiness were run. In Simulation 1, beetles were less likely to mate heterospecifically upon contact as they became rarer. In Simulation 2, beetles were more likely to mate conspecifically, but choosiness did not vary with rarity. In Simulation 3, the probability of heterospecific mating increased as rarity decreased, while the probability of conspecific mating decreased. Finally, in Simulation 4, beetles were more likely to mate heterospecifically as they became rarer. Using pair type frequencies obtained through these simulations, I_{PSI} was calculated to find which simulation produced a relationship between I_{PSI} and relative abundance that corresponded best to the results from our experiment.

Results

Objective 1: Are multi-choice experiments in the lab representative of nature?

Among the three replicate trials with beetles from site S at a 50:50 species ratio, there was no significant effect of replicate for either probability of heterospecific mating ($F_{2,124}=1.148$, p=0.32), PSI ($F_{2,99}=0.15$, p=0.99) or overall isolation ($F_{2,42}=0.585$, p=0.56). Based on this result, it appears that the results from a multi-choice experiment using any group of 100 beetles from a population can be assumed to be representative of that population. Results from the trial using site S beetles at a ratio of 30 *C. cobaltinus* : 70 *C. auratus* were similar to previous field data from that site (Table 1). For both data sets, PSI estimates for the conspecific pairs were greater than 1, while PSI estimates for heterospecific pairs were lower than 1, indicating assortative mating (Rolán-Alvarez & Caballero 2000), Furthermore, PSI values for *C. cobaltinus* pairs and *C. cobaltinus* male

x *C. auratus* female pairs fell within the error terms; although this was not the case for *C. auratus* pairs and *C. auratus* male x *C. cobaltinus* female pairs, the PSI values were still fairly close (Table 1). The error term used for the lab data is standard error, calculated across replicate observations. Only one observation was taken for the field data, so a standard error could not be calculated. Instead, the error term for the field data is a bootstrapped estimate of standard deviation.

Objective 2: Does sexual isolation vary among populations in the hybrid zone?

No significant differences in any PSI values were found between populations AF, AR and S, for trials conducted at a 50:50 ratio of the two species ($F_{2,123}=0.044$, $p=0.96$). This result shows that sexual isolation does not differ among the three populations sampled.

Objective 3: Does the risk of hybridization depend on relative abundance?

Relative abundance had a significant influence on a beetle's risk of hybridization. For both sexes of both species, as a beetle species became rarer it had a greater risk of mating with a heterospecific individual instead of a conspecific individual ($F_{4,220}=18.216$, $p<0.01$) (Figure 1). Generally speaking, when a species was the relatively abundant species (70% or 90% of individuals), the probability of mating with a heterospecific individual was consistent with random mating. In contrast, with the exception of *C. auratus* females (at 10% of individuals), when a species was rare (10% or 30% of individuals), they mated with heterospecific individuals less than expected by chance (Figure 1).

Objective 4: Does relative abundance influence patterns of sexual isolation?

Relative abundance also had a significant effect on sexual isolation. While PSI values were above 1 for conspecific pairs and below 1 for heterospecific pairs in all relative abundances, missing data points in both extreme populations (90:10 and 10:90) caused data reliability to be suspect, as the PSI values could either be caused by true sexual isolation or lack of data. Thus, we report herein only the relationship between overall sexual isolation (I_{PSI}) and relative abundance. As the relative abundance of the two species ratios became more divergent (i.e. one species is getting rarer), overall sexual isolation increased ($F_{4,70}=0.927$, $p=0.45$) (Figure 2, Lab).

Objective 5: Does choosiness vary with relative abundance?

In order to examine why overall sexual isolation values are greatest when relative abundances are most divergent, we compared our multi-choice experiment results with the results from our simulations in which choosiness of the beetles were varied. The simulation which most closely resembled the curve shape given by laboratory data was one in which a species gets choosier as it gets rarer (Figure 3). Although the overall level of sexual isolation differed between this simulation and the lab results, the pattern of increasing sexual isolation with increasing differences in relative abundance was repeated with this simulation (Figure 2). In contrast, overall sexual isolation did not vary substantially with relative abundance when choosiness was assumed to not vary with relative abundance (Figure 2), or with the other simulations.

Discussion

The consistency of results from the three trials of population S run at a 50:50 species ratio indicate that any group of beetles selected for testing can be considered representative of their population. The fact that two of four possible mating combinations

fell within comparative PSI value error bounds, while the other two possibilities fell quite close, gives us a reasonable assurance that the multi-choice experiments conducted in lab are representative of mate choices observed in nature. Most importantly, in both lab and field, PSI values for conspecific pairs were greater than 1, while those for heterospecific pairs were less than 1, a pattern indicative of sexual isolation (Rolán-Alvarez & Cabellero, 2000). No significant differences in sexual isolation were found among any of the factors tested between populations AF, AR, and S, indicating that sexual isolation does not vary significantly among populations in the hybrid zone. Thus, it appears that sexual isolation does not differ genetically among these populations.

The experiments in which species ratio was adjusted showed a clear relationship between relative abundance and the risk of hybridization; as relative abundance was lowered, the risk increased. This makes sense in that when a species is rare, it is less likely to encounter a mate of its own species. For example, when one species comprises only 10% of the beetles, there is only a 5% chance that a beetle of the opposite sex is of their species, and thus the rare species mates more often with heterospecific individuals than does the common species. Relative abundance also significantly affected overall sexual isolation (I_{PSI}). I_{PSI} values were constantly greater than zero, indicating sexual isolation at all combinations of relative abundance. However, sexual isolation clearly increased when either species became rare. The evidence that sexual isolation increases with rarity seems at odds with the increasing risk of hybridization. If rare beetles are more isolated and are more biased toward conspecific mating, it seems that they should be less at risk of hybridization. To give insight into this paradox, we performed various isolation simulations, varying choosiness of individuals in relation to relative abundance

to determine how varying choosiness might influence I_{PSI} (data not shown). It was clear upon comparison that the distinctive relationship between I_{PSI} and relative abundance seen in the lab was consistent with beetles becoming conspecifically choosy when selecting mates as their own species becomes rarer. This result is consistent with our finding that individuals were less likely to hybridize then by chance at low, but not high relative abundances (Figure1). Our results support the assumption that hybridization risk increases with relative scarcity in a hybrid zone, but also reveal that relative abundance alone may overestimate the risk of hybridization, if choosiness also increases with increasing scarcity.

Now that lab results have been shown to be representative of nature, future research should be conducted in the field to assess the relative abundance of naturally occurring populations within the hybrid zone and whether relative abundance is linked to the risk of hybridization similarly to what was observed in lab. If so, populations should have evolved different degrees of choosiness. This expectation is due to differences in selective pressures throughout the zone based on relative abundance, and the proximity of populations in the hybrid zone to parental populations. Proximity to parental populations affects the degree of gene flow which counteracts the evolution of reproductively isolating trait differences among hybrid zone populations (Howard, 1993). Populations at either extreme in the hybrid zone as well as in the center could be assessed to give evidence for the hypothesis that the degree of choosiness will increase with distance from the parental population such that gene flow is lessened.
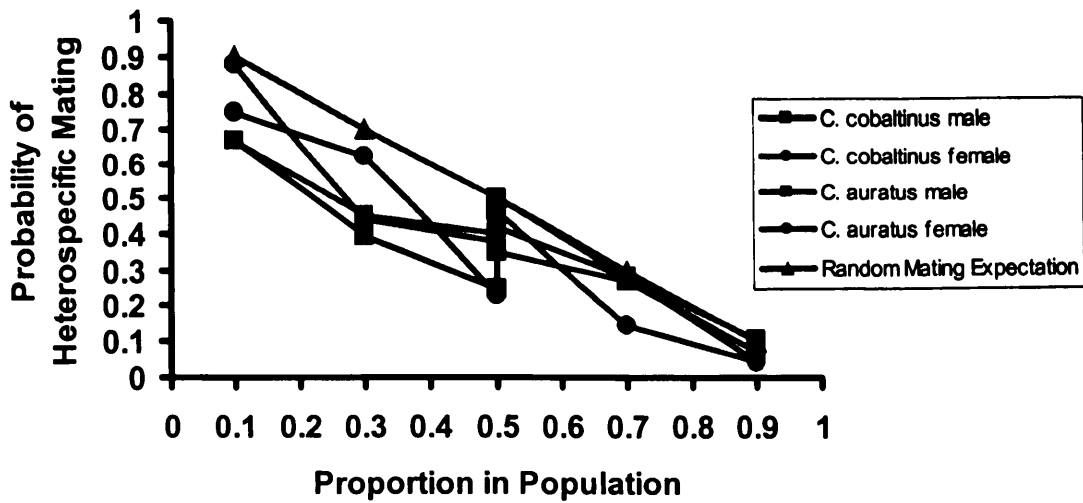
Figure 1. The risk of heterospecific mating in relation to relative abundance. Both species and sexes showed a greater risk of hybridization when rare.
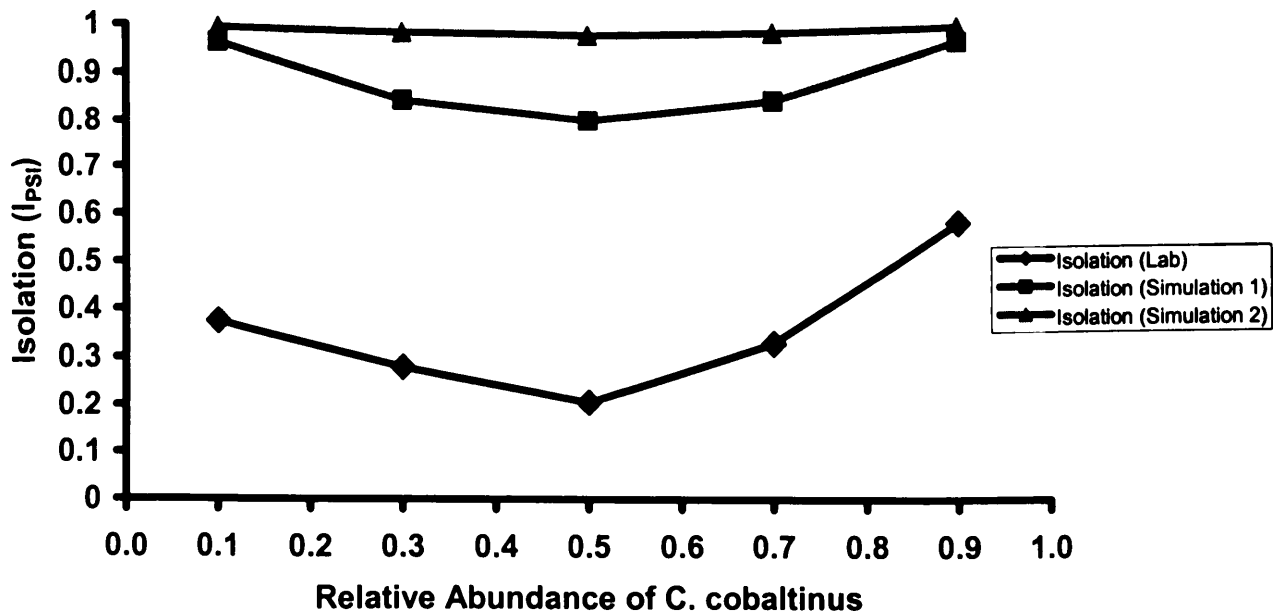

Figure 2. The effect of relative abundance on overall sexual isolation (I_{PSI}) between *C. cobaltinus* and *C. auratus*. Similar to the results for the multi-choice experiments (Lab), sexual isolation peaked at increasing divergence of relative abundance for the simulation in which choosiness was assumed to increase with increasing rarity (Simulation 1). This

was not true if choosiness was assumed to be constant across all relative abundances (Simulation 2)
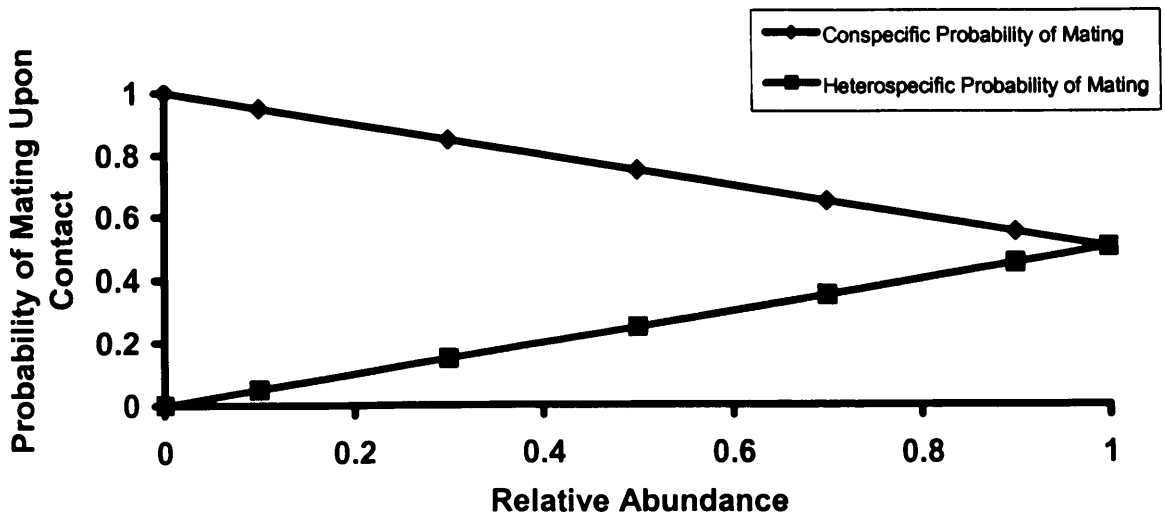

Figure 3. Simulation 1, which resulted in an isolation curve most resembling data obtained in lab. Under this simulation, individuals become choosier as the relative abundance of their species declines.

Table 1. PSI values for field data at Site S, and lab data from a cage run at 30 *C. cobaltinus* : 70 *C. auratus*. This ratio most closely approximates the species ratio of the field population. The error term for the field data is a bootstrapped estimate of standard deviation; lab results use standard error. Values for *C. cobaltinus* pairs and *C. cobaltinus* male x *C. auratus* female pairs both fall within the error terms.

	C. cobaltinus pair	*C. cobaltinus* male x *C. auratus* female	*C. auratus* pair	*C. auratus* male x *C. cobaltinus* female
Field Data	1.325 ± 0.108	0.535 ± 0.130	1.550 ± 0.176	0.626 ± 0.109
Lab Data	1.479 ± 0.304	0.616 ± 0.161	1.120 ± 0.051	0.806 ± 0.110

Literature Cited

Coyne J.A. (1992). Genetics and speciation. *Nature*, *355*, 511-515.

Coyne, J.A., Kim, S.A., Chang, A.S., Lachaise, D., and Elwyn, S. (2002). Sexual isolation between two sibling species with overlapping ranges: *Drosophila santomea* and *Drosophila yakuba*. *Evolution*, *56*, 2424-2434.

Dobzhansky, T. (1940). Speciation as a stage in evolutionary divergence. *American Naturalist*, *74*, 312-321.

Gilbert, D.G., and Starmer, W.T. (1985). Statistics of sexual isolation. *Evolution*, *39*, 1380-1383.

Harrison, R.G. (ed.). (1993). *Hybrid Zones and the Evolutionary Process*. Oxford University Press: New York.

Howard, D.J. (1993). Reinforcement: origin, dynamics and fate of an evolutionary hypothesis. In Harrison, R.G. (ed.), *Hybrid Zones and the Evolutionary Process*, (pp.46-69). Oxford University Press: New York.

Mayr, E. (1970). *Populations, Species, and Evolution. An Abridgement of Animal Species and Evolution*. Harvard University Press: Cambridge.

McLain, D.K. (1985). Clinal variation in morphology and assortative mating in the soldier beetle, *Chauliognathus pennsylvanicus* (Coleoptera: Centhanidae). *Biological Journal of the Linnean Society*, *25*, 105-117.

Nosil, P., Crespi, B.J. and Sandoval, C.P. (2003). Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proceedings of the Royal Society of London B*, *270*, 1911-1918.

Peterson, M.A., Dobler, S., Holland, J., Tantalo, L. and Locke, S. (2001). Behavioral, molecular and morphological evidence for a hybrid zone between *Chrysochus auratus* and *C. cobaltinus* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America*, *94*, 1-9.

Peterson, M.A., Monsen, K.J., Pedersen, H., McFarland, T., and Bearden, J. (in press). Direct and indirect analysis of the fitness of *Chrysochus* (Coleoptera: Chrysomelidae) hybrids. *Biological Journal of the Linnean Society*.

Ribi, G., and Oertli, S. (2000). Frequency of interspecific matings and of hybrid offspring in sympatric populations of *Viviparus ater* and *V. contectus* (Mollusca: Prosobranchia). *Biological Journal of the Linnean Society*, *71*, 133-143.

Rolán-Alvarez, E., Erlandsson, J., Johannesson, K., and Cruz, R. (1999). Mechanisms of incomplete prezygotic reproductive isolation in an intertidal snail: testing behavioral models in wild populations. *Journal of Evolutionary Biology, 12*, 879-890

Rolán-Alvarez, E. and Caballero, A. (2000). Estimating sexual isolation and sexual isolation effects from mating frequencies. *Evolution, 54*, 30-36.

Servedio, M.R. and Noor, M.A.F. (2003). The role of reinforcement in speciation: Theory and data. *Annual Review of Ecology, Evolution and Systematics, 34*, 336-364.