Winter 2000

Reproductive Character Displacement and X-linkage of Cuticular Hydrocarbons in Chrysochus beetles

Hallie Kerins
Western Washington University

Honors Program

HONORS THESIS

In presenting this Honors paper in partial requirements for a bachelor's degree at Western Washington University, I agree that the Library shall make its copies freely available for inspection. I further agree that extensive copying of this thesis is allowable only for scholarly purposes. It is understood that any publication of this thesis for commercial purposes or for financial gain shall not be allowed without my written permission.

Signature [redacted]

Date 3/20/06

Reproductive Character Displacement and X-linkage of Cuticular Hydrocarbons in *Chrysochus* beetles

Hallie Kerins
Biology Department, Western Washington University

Abstract

Reproductive isolation has been one major focus of current speciation research with two major lines of investigation: 1) the mechanisms and evolution of behavioral isolation and, 2) the genetic changes underlying reproductive isolation. The reproductive isolation between closely related insect species often involves chemical signaling systems, with one example being cuticular hydrocarbons (CHCs). The divergence in the chemical signaling systems is important in the evolution of pre-mating barriers in insects, thus CHCs may play an important role in the evolution of reproductive barriers. Behavioral divergence between closely related species is common and studies are beginning to show that these behavioral differences that result in species isolation have a genetic basis. We examined the evolution and genetic basis of reproductive isolation, using *Chrysochus* leaf beetles as a model system. Comparing CHC profiles, we assessed whether CHC profiles exhibit reproductive character displacement and whether CHC profiles are X-linked. Some aspects of CHC profiles were consistent with reproductive character displacement, while others were not. In the absence of knowledge regarding which specific CHCs govern mate choice, it is premature to determine if those key CHCs exhibit reproductive character displacement. There was no evidence to support the hypothesis of X-linkage of CHC profiles. These results will provide an important context for interpreting future studies on the evolution of mating cues in this system.

Introduction

Current speciation research is attempting to understand the processes that drive the evolution of new species. Reproductive isolation has been the focus of much of this study with two major lines of research investigating 1) the mechanisms and evolution of behavioral isolation (e.g. Higgie 2000; Peterson 2005; Ptacek 2001) and, 2) the genetic changes underlying reproductive isolation (e.g. Counterman et al. 2004; Dopman 2004; Orr 2001). The focus on reproductive isolation stems from the important role it plays in the biological species concept, which states that organisms are classified in the same species if they are potentially capable of interbreeding and producing fertile offspring

(Coyne 1992). So, the start of a new species involves the evolution of barriers to gene exchange between diverging populations (Dopman 2004). In sympatric zones, or overlapping geographic regions, two incipient species that were once separated by a barrier, such as a mountain range or river, might come into secondary contact. If these two species attempt to interbreed and produce offspring, a hybrid zone may form. This is one situation in which evolution is a dynamic process and it is possible to study the process using hybrid zones. If the hybrids have lower fitness than either parental form, selection should favor the evolution of reproductive isolation by favoring genes which allow individuals to avoid mating with the wrong species (Dobzhansky 1940). Reinforcement is this process by which natural selection works against hybridization to increase reproductive isolation between two closely related species in areas of secondary contact. The expected consequence of reinforcement would be greater divergence of species in places where hybridization is occurring than in areas where the species do not come into contact, a pattern known as reproductive character displacement (Howard 1993).

Reinforcement theory is one of the most frequently studied and controversial theories of speciation, and support for the theory has been variable (Noor 1999). Enthusiasm for the theory was initially high following work done by Dobzhansky (1940), but in the 1980s, acceptance fell drastically due to theoretical and verbal arguments against it, which suggested that very strong selection would be required to compensate for the negative effects of recombination and gene flow (Paterson 1982). Acceptance for reinforcement rose again with both theoretical and empirical support. Coyne and Orr (1989) showed that sympatric species pairs tend to exhibit stronger species mating

discrimination, than allopatric species pairs of the same genetic divergence. Now, the process of reinforcement is widely accepted (Servedio et al. 2003).

In studying the evolution of reproductive barriers, research has focused on characters associated with morphological and behavioral isolation. The reproductive isolation between closely related insect species often involves chemical signaling systems (Roelofs & Comeau 1969; Cardé et al. 1977). Cuticular hydrocarbons (CHCs), together with chitin, are the principle cuticular compound in most insects and are frequently used for intra- and interspecific communication, particularly mate recognition (Blomquist et al. 1998). The divergence in the chemical signaling systems is important in the evolution of pre-mating barriers in insects (Howard 1993), thus CHCs may play an important role in the evolution of reproductive barriers (Higgie et al. 2000). Higgie et al. (2000) exposed field sympatric and allopatric populations of *Drosophila serrata* to experimental sympatry with *D. birchii*. They found that CHCs of field allopatric *D. serrata* populations evolved to resemble the field sympatric populations, whereas field sympatric *D. serrata* populations remained unchanged. In this study, natural selection on mate recognition resulted in reproductive character displacement. However, because very few studies have examined the idea of pre-mating barriers with respect to CHCs, it is unknown whether or not the Higgie et al. (2000) study is representative of the typical process by which CHC differences arise. Studying the divergence of chemical signaling in other systems could add to our understanding of speciation. The purpose of the research in this paper is to determine if the CHC profiles of hybridizing *Chrysochus* beetles exhibit geographic variation, or show differences between allopatric and

sympatric individuals, that match behavioral evidence for reproductive character displacement.

Behavioral divergence between closely related species is common and studies are beginning to show that these behavioral differences that result in species isolation have a genetic basis (Ptacek 2002). Research on the genetic basis for speciation has focused on the identification and characterization of the genetic elements that contribute to reproductive barriers (Dopman 2004). Results to date suggest that very few genes underlie the phenotypic differences involved in speciation (Orr 2001) and there is evidence to suggest these genes are often X-linked (Counterman et al. 2004). A genetic map could assist in the isolation of specific genes involved in sexual isolation, as many reproductive barriers have a complex, polygenic basis (Coyne and Orr 1998). Several studies have shown that pheromone production involves only five or six genes and is autosomally inherited (Orr 2001). Similarly, the male courtship traits, such as courtship songs, involve only two major genes (Orr 2001) and seem to be sex-linked, specifically maternally inherited via sex chromosomes (Counterman et al. 2004; Klun and Maini 1979; Roelofs et al. 1987; Glover et al. 1990). While progress is being made, more studies are needed if we are to be able to order to draw general patterns.

The evolution and genetic basis of reproductive isolation can be studied using the leaf beetles, *Chrysochus cobaltinus* and *C. auratus* (Coleoptera: Chrysomelidae) as a model system. They hybridize in nature, forming a hybrid zone in the Yakima River Valley of south-central Washington (U.S.A.) (Peterson et al. 2001). Both males and females mate daily throughout their 6-8 week lifespan (Dickinson 1995, 1997). While the hybrids engage in copulatory behavior, they are effectively sterile (Peterson et al.

2005). The parentals show positive assortative mating, or selective mating in which members of the same species mate more frequently with one another than predicted by chance. This assortative mating is due, at least in part, to the response of males to species- and sex-specific CHC profiles, which feature an array of long, straight chain hydrocarbons with very little unsaturation (Peterson et al. in review). Having a species in which a single reproductive character is largely responsible for mate recognition provides a good system for studying reproductive character displacement (Blair 1964). Evidence of reproductive character displacement, supports the hypothesis of reinforcement in this system (Peterson et al. 2005). Specifically, *C. cobaltinus* males from the hybrid zone are choosier than males from outside the hybrid zone, and appear better able to distinguish hybrid zone females than non-hybrid zone females. However, it remains unresolved whether this pattern of reproductive character displacement is due in part to greater divergence of CHC profiles inside the hybrid zone than outside of it.

This Chrysochus system can also be used to examine the genetic changes involved in reproductive isolation. Examining the differences between hybrids and *C. cobaltinus* and *C. auratus* sympatric populations can be used to test the hypothesis that hydrocarbon profiles are inherited maternally. Species specific mitochondrial markers have shown that the vast majority (~90%) of hybrid individuals are born from *C. cobaltinus* females (Monsen et al. in review). This strong bias in the directionality of hybrids provides an opportunity to address the idea that the X-chromosome plays an important role in sexual selection in diverging populations (Counterman et al. 2004). If CHCs are maternally inherited in this system, the CHC profiles of hybrid males would be

more similar to those of *C. cobaltinus* males, and the CHC profiles of hybrid females would be intermediate between those of *C. cobaltinus* and *C. auratus* females.

To test the hypothesis that reproductive character displacement could be seen in CHCs, we compared allopatric *C. cobaltinus* and *C. auratus* to sympatric *C. cobaltinus* and *C. auratus* populations. To test the hypothesis that CHC profiles are X-linked (Counterman et al. 2004), we compared the profiles of sympatric *C. cobaltinus* and *C. auratus* to those of hybrid beetles.

Materials and Methods

In this study, data were collected on CHC profiles for 60 sympatric individuals (10 males and 10 females of each *C. cobaltinus*, *C. auratus*, and hybrids). All sympatric beetles were taken from the focal hybrid zone population used in other studies (Peterson et al. 2005). These data were compared to data collected and analyzed by Peterson et al. (in review) for 40 allopatric individuals outside the hybrid zone, including 11 female and nine male *C. cobaltinus* and 10 female and 10 male *C. auratus*.

Following protocols used in Peterson et al. (in review), hexane washes (10 sec/beetle in 1 mL hexane) were used to obtain cuticular extracts from each individual. The extracts were then put through silica column chromatography for purification, after which an internal standard of n-hexacosane (5 ng/μl) was added in order to align the peaks across each run before being analyzed by gas chromatography (GC). We performed GC with an HP 6890 (Hewlett Packard, Palo Alto, CA, USA), using a 30 m, 0.25 mm id fused silica capillary CP-Sil 8CB (Varian, Darmstadt, Germany) with the temperature programmed from 150-300 °C at a rate of 10 °C/min.

Before data analysis, we used the internal standard, n-hexacosane, to align the GC peaks across all individuals in order to adjust for small differences in retention times across sample runs. To be consistent for comparison purposes, we used only those peaks that had been analyzed in the earlier allopatric study done by Peterson et al. (in review). In that study, the only peaks analyzed were those present in all members of at least one sex of one species and represented at least 1% of the average total peak area of at least one sex of one species. For the present study, we also excluded two peaks (15,19-dimethyltritriacontane and n-tetratriacontane) that had been in the early study but were missing from many sympatric individuals. These peaks have long retention times, and it appeared that run times for some sympatric individuals had been truncated relative to those in the allopatric study. The same two peaks were also removed from the allopatric data and the data were reanalyzed so comparisons could be made.

For the remaining twenty-four peaks, we performed analyses based on a protocol used by Neems and Butlin (1995), the same protocol used for the allopatric studies (Peterson et al. in review). We calculated the relative peak areas based on the total area of only the included peaks and then converted the relative peak areas to log contrasts. The log contrast approach allows for later multivariate analysis (Neems & Butlin 1995), and it involves dividing the relative peak area of each peak by the peak area of the arbitrarily chosen peak, n-octacosane in this case, and then taking the log of these new ratios (Simmons et al. 2003).

Principal components analysis was then performed on the log contrasts to reduce the variables to a smaller set of principal components. This analysis was conducted using SPSS 11.0.1 (SPSS Inc., 2003) using varimax rotation with Kaiser normalization, with

the cutoff for extraction being an eigenvalue of at least 1.0. The principal components scores for all individuals were then subjected to MANOVA to determine the overall influence of sex, species, geography and their two- and three-way interactions on the principal component scores. We then ran ANOVAs to determine which principal components were influenced by sex, species, geography, and their various interactions. Pearson correlation coefficients between each principal component and the relative areas of each peak were estimated to determine which peaks contributed a significant amount to each principal component. Peaks were considered to be significan contributors of principal components based on recommendations from Mardia et al. (1979). Specifically, if a peak exceeded 70% of the largest pairwise correlation coefficients between peaks and the principal component, then it was considered a significant contributor to the principal component.

Results

Reproductive Character Displacement of CHC Profiles

Principal components analysis of allopatric and sympatric parentals resulted in six principal components (PC), which together explained 80.47% of the variation among samples. PCs 1-6 accounted for 21.9, 16.4, 12.8, 12.2, 9.9, and 7.3% of the total variance, respectively. MANOVA of these PCs revealed significant effects of species, sex, geography, and all two- and three-way interactions on cuticular chemistry profiles (Table 1). ANOVA for each PC showed that different overlapping subsets of the six PCs were influenced by difference among species, sex, geography, species/sex interaction,

species/geography interaction, sex/geography interaction, and species/sex/geography interaction (Table 2).

Correlations between relative peak areas and PCs 1-6 indicate which compounds were significant contributors to those PCs (Table 3). Relative peak areas for each compound are provided in table 4 to facilitate the interpretation of correlations. PC1, which was influenced by species, sex, geography, and a species/sex interaction, was correlated with n-triacontane, 2-methyltriacontane, 9-hentriacontene, n-hentriacontane, n-dotriacontane, 2-methyldotriacontane, and n-tritriacontane. PC2, which was influenced by sex, geography, a species/sex interaction, a sex/geography interaction, and a species/sex/geography interaction, was correlated with n-docosane, 2-methyldocosane, 9-tricosene, and n-tricosane. PC3, which was influenced by a species/geography interaction and a sex/geography interaction, was correlated with 2-methyltetracosane, 9-pentacosene, and n-pentacosene. PC4, which was influenced by species, geography, a species/sex interaction, and a species/geography interaction, was correlated with 9-nonacosene and the peak containing both 13-methylhentriacontane and 15-methylhentriacontane. PC 5, which was influenced by sex, a species/sex interaction, and a species/geography interaction, was correlated with n-heptacosane, 2-methyloctacosane, and n-nonacosane. PC 6, which was influenced by species, geography, a species/sex interaction, and a species/sex/geography interaction, was correlated with 2-methylhexacosane.

The PCA results provide variable evidence for reproductive character displacement. For example, while principal components plots suggest greater divergence in sympatery (Figure 1) others show no such pattern of divergence (Figure 2).

Are CHC Profiles X-linked?

Principal components analysis of sympatric parentals and hybrids resulted in 6 PCs which together explained 85.56% of the total variation among samples. PCs 1-6 accounted for 21.3, 18.1, 16.2, 13.9, 8.1, and 8.0% of the total variance, respectively. MANOVA of these PCs revealed significant effects of species, sex, and a species/sex interaction on cuticular chemistry profiles (Table 5). ANOVA for each PC showed that different overlapping subsets of the six PCs were influenced by differences among species, sex, and the species/sex interaction (Table 6).

Correlations between relative peak areas and PCs 1-6 indicate which compounds were significant contributors to each PC (Table 7). Relative peak areas for each compound are provided in table 4 to facilitate the interpretation of correlations. PC1, which was influenced by sex, was correlated with 2-methyldocosane, 9-tricosene, n-tricosane, n-triacontane, and 9-hentriacontene. PC2, which was influenced by sex, was correlated with 2-methyltetracosane, 9-pentacosene, n-pentacosane, and n-heptacosane. PC3, which was influenced by sex, was correlated with 2-methyltriacontane, n-hentriacontane, n-dotriacontane, 2-methyldotriacontane, and n-tritriacontane. PC4, which was influenced by species, was correlated with 2-methylheptacosane and 2-methyloctacosane. PC5, which was influenced by species and a species/sex interaction, was correlated with 9-nonacosene and the peak including both 13-methylhentriacontane and 15-methylhentriacontane. PC6, which was influenced by species and the species/sex interaction, was correlated with peaks n-docosane, and n-tetracosane.

In general, when hybrids were more similar to one parent than the other, they were similar to *C. auratus*. With respect to parentals, hybrid males tend to cluster with *C.*

auratus (Figure 3 and Figure 4) and hybrid females show clustering with *C. auratus* (Figure 5 and Figure 6) on PC plots. Cluster analysis revealed similar patterns (Figure 7). Female hybrids tend to cluster with *C. auratus* females more often than with *C. cobaltinus* females. When hybrid females do cluster with *C. cobaltinus*, there is also clustering with *C. cobaltinus* males, *C. auratus* males, and hybrid males, making it difficult to determine which individuals actually exhibit the most similarities. Male hybrids also tend to cluster with *C. auratus* more often than with *C. cobaltinus*.

Discussion

Is Reproductive Character Displacement Seen In CHC Profiles?

Based on results of Peterson et al. (2005), it is known that *C. cobaltinus* males are better able to differentiate between *C. cobaltinus* and *C. auratus* females, and choose the female of its own species if the females are from the hybrid zone. This suggests that *C. cobaltinus* females within the hybrid zone should have more distinguishable CHC profiles in sympatry, as has been seen in other systems (Higgie 2000). From this prediction, we would expect allopatric *C. cobaltinus* and *C. auratus* to show more overlap on a PC plot than sympatric *C. cobaltinus* and *C. auratus*. Although we found such a pattern from some PCs, this pattern was far from universal.

Thus, at present we cannot tell whether cues for mating are more or less divergent between allopatric and sympatric populations. If there was significant divergence, the principal component plots strongly influenced by the peaks involved in mate selection would show distinct clustering with little overlap between *C. auratus* and *C. cobaltinus*. However, the peaks analyzed here were chosen because they show up in all members of

at least one sex of one species and represented at least 1% of the average total peak area of at least one sex of one species, not based on their importance in mate selection. We would need to determine which specific compounds are important for mating divergence in order to determine whether or not there is a significant difference in the mating cues between allopatric and sympatric populations. To do this would require additional bioassays or electrophysiological studies.

Are CHC Profiles X-linked?

Previous studies have shown that very few genes are involved in mate selection (Orr 2001) and a high proportion of genes involving hybrid sterility are found on the X-chromosome (Counterman et al. 2004). Comparing *C. cobaltinus* and *C. auratus* sympatric and hybrid populations can be used as a loose test of the hypothesis that hydrocarbon profiles are X-linked. Our analysis showed no evidence to support this hypothesis. Based on mitochondrial DNA, we know that 90% of hybrids are a result of mating between *C. auratus* male and a *C. cobaltinus* female (Peterson et al. 2005). Therefore, most male hybrid offspring would have received their X-chromosome from *C. cobaltinus* and Y-chromosome from *C. auratus*. If CHCs were X-linked, we would expect the CHC profiles of hybrid males to be more similar to those of *C. cobaltinus* males. Similarly, because a female offspring would receive an X-chromosome from each parent, we would expect the CHC profile of hybrid females to be intermediate between those of *C. cobaltinus* and *C. auratus* females. Such patterns are not seen in our results. As stated previously, though, we do not know which peaks are involved in mate selection and therefore we do not know which principal components are strongly influenced by mate selection peaks. Therefore, we do not know if the clustering of hybrids with *C.*

auratus has to do with mate selection peaks or other peaks. Nonetheless, having solid baseline comparisons between hybrids and parentals and between sympatric and allopatric populations provides an important context for interpreting future studies that identify which peaks are important in mate recognition. Many studies have looked at cues influencing isolation (e.g. Higgie 2000; Peterson 2005; Ptacek 2001) and the genetic basis of species differences (e.g. Counterman 2004; Dopman 2004; Orr 2001) but few have tied both concepts together. This study is an important step in that direction.

Works Cited

Blair, W.F. 1964. Isolating Mechanisms and Interspecies Interactions in Anuran Amphibians. Q. Rev. Biol. 39: 334.

Blomquist, G.J., J.A. Tillman, S. Mpuru, and S.J. Seybold. 1998. The cuticle and cuticular hydrocarbons of insects: structure, function, and biochemistry. Pp. 34-54 *in* R.K. Vander Meer, M. Breed, M. Winston, and C. Espelie, eds. Pheromone Communication in Social Insects. Westview Press, Bouldor, CO.

Cardé, R.T., C.C, Doane, T.C. Baker, S. Iwaki, and S. Marumo. 1977. Attractancy of optically active pheromone for male gypsy moths. Environ. Entomol. 6: 768-772.

Counterman, M.A., D. Ortiz-Barrientos, and M.A.F. Noor. 2004. Using comparative genomic data to test for fast-x evolution. Evolution 58(3): 656-660.

Coyne, J.A. 1992. Genetics and speciation. Nature 355: 511-515.

Coyne, J.A. and H.A. Orr. 1998. The evolutionary genetics of speciation. Phil. Trans. R. Soc. Lond. B. 353: 287-305.

Coyne, J.A. and H.A. Orr. 1989. Patterns of speciation in *Drosophila*. Evolution 43: 362-381.

Dickinson, J.L. 1995. Trade-offs between postcopulatory riding and mate location in the blue milkweed beetle. Behavioral Ecology 6: 280-286.

Dickinson, J.L. 1997. Multiple mating, sperm completion and cryptic female choice in the leave beetles (Coleoptera: Chrysomelidae) Pp. 164-183. *in* J.C. Choe, B.J., Crepsi, eds. The evolution of mating systems in insects and arachnids. Cambridge: Cambridge Univ. Press.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. 74: 312-321.

Dopman, E.B., S.M. Bogdanowicz, and R.G. Harrison. 2004. Genetic mapping of sexual isolation between E and Z pheromone strains of the European Corn Borer (*Ostrinia nubilalis*). Genetics 167: 301-309.

Glover, T., M. Campbell, P. Robbins, and W. Roelofs. 1990. Sex linked control of sex pheromone behavior responses in European corn borer moths, *Ostrinia nubilalis*, confirmed with TPI marker gene. Archives of Insect Biochemistry and Physiology 15: 67-77.

Higgie, M., S. Chenoweth, and M.W. Blows. 2000. Natural selection and the reinforcement of mate recognition. Science 290: 519-521.

Howard, D.J. 1993. Reinforcement: origin, dynamics and fate of an evolutionary hypothesis. Pp. 46-69 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, Oxford, U.K.

Klun, J.A., S. Maini. 1979. Genetic basis of an insect communication system: the European corn borer. Environ. Entomol. 8: 423-426.

Mardia, K.V., J.T. Kent, and J.M. Bibby. 1979. Multivariate Analysis. London: Academic Press.

Monsen, K.J., B.M Honchak, S.E. Locke, M.A. Peterson. *In review*. Cytonuclear disequilibrium in the *Chrysochus* hybrid zone. Molecular Ecology.

Neems, R.M., and R.K. Butlin. 1995. Divergence in cuticular hydrocarbons between parapatric subspecies of the meadow grasshopper, Chorthippus parallelus (Orthoptera, Acrididae). Biological Journal of the Linnean Society 54(2): 139-149.

Noor, M.A. 1999. Reinforcement and other consequences of sympatry. Heredity 83: 503-508.

Orr, H.A. 2001. The genetics of species differences. Trends in Ecology & Evolution 16(7): 343-350.

Paterson, H.E.H. 1982. Perspective on speciation by reinforcement. S. Afr. J. Sci. 78: 53-57.

Peterson, M.A., S. Dobler, J. Holland, L. Tantalo, and S. Locke. 2001. Behavioral, molecular, and morphological evidence for a hybrid between *Chrysochus auratus* and *C. cobaltinus* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 94(1): 1-9.

Peterson, M.A., B.M. Honchak, S.E. Locke, T.E. Beeman, J. Mendoza, J. Green, K.J. Buckingham, M.A. White, and K.J. Monsen. 2005. Relative abundance and the species-specific reinforcement of male mating preference in the *Chrysochus* (Coleoptera: Chrysomelidae) hybrid zone. Evolution 59(12): 2639-2655.

Peterson, M.A., K.J. Monsen, H. Pedersen, T. McFarland, and J. Bearden. 2005. Direct and indirect analysis of the fitness of *Chrysochus* (Coleoptera: Chrysomelidae) hybrids. Biolgical Journal of the Linnean Society 84: 273-286.

Peterson, M.A., S. Dobler, E.L. Larson, D. Juarex, T. Schlarbaum, K.J. Monsen, and W. Francke. *In review*. Profiles of cuticular hydrocarbons mediate male mate choice and sexual isolation between hybridizing *Chrysochus* beetles. Animal Behavior.

Ptacek, M.B. 2002. Patterns of inheritance of mating signals in interspecific hybrids between sailfin and shortfin mollies (Poeciliidae: *Poecilia: Mollienesia*). Genetica 116: 329-342.

Roelofs, W.L. and A. Comeau. 1969. Sex pheromone specificity—taxonomic and evolutionary. Science 165: 398-400.

Roelofs, W.L., T. Glover, X.H. Tang, I. Streng, P. Robbins, C. Eckenrode, C. Loefstedt, B.S. Hansson, B.O. Bengtsson. 1987. Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. Proceedings of the National Academy of Sciences, USA 84: 7585-7589.

Servedio, M.R. and M.A.F. Noor. 2003. The role of reinforcement in speciation: theory and data. Annual Review of Ecology, Evolution and Systematics 34: 339-364.

Simmons, L.W., J. Alcock, and A. Reeder. 2003. The role of cuticular hydrocarbons in male attraction and repulsion by female Dawson's burrowing Bee, *Amegilla dawson*. Animal Behavior 66: 677-685.

Table 1: MANOVA of PCs for *C. cobaltinus* and *C. auratus* allopatric and sympatric populations.

Source	F-value	p-value
Species	$F_{6,67} = 20.01$	$p < 0.001$
Sex	$F_{6,67} = 34.54$	$p < 0.001$
Geography	$F_{6,67} = 52.04$	$p < 0.001$
Species x Sex	$F_{6,67} = 12.11$	$p < 0.001$
Sex x Geography	$F_{6,67} = 7.51$	$p < 0.001$
Sex x Geography	$F_{6,67} = 5.41$	$p < 0.001$
Species x Sex x Geography	$F_{6,67} = 2.24$	$p < 0.001$

Table 2: ANOVA values for *C. cobaltinus* and *C. auratus* allopatric and sympatric populations. F- and p-values indicate which source influenced each PC.

Source	PC1	PC2	PC3	PC4	PC5	PC6
Species	$F_{1,72} = 52.25$ ($p < 0.001$)	$F_{1,72} = 1.61$ ($p = 0.285$)	$F_{1,72} = 2.24$ ($p = 0.138$)	$F_{1,72} = 37.12$ ($p < 0.001$)	$F_{1,72} = 0.62$ ($p = 0.435$)	$F_{1,72} = 7.69$ ($p = 0.007$)
Sex	$F_{1,72} = 33.4$ ($p < 0.001$)	$F_{1,72} = 72.27$ ($p < 0.001$)	$F_{1,72} = 0.72$ ($p = 0.400$)	$F_{1,72} = 2.59$ ($p = 0.112$)	$F_{1,72} = 9.76$ ($p = 0.003$)	$F_{1,72} = 1.12$ ($p = 0.294$)
Geography	$F_{1,72} = 75.37$ ($p < 0.001$)	$F_{1,72} = 10.49$ ($p = 0.002$)	$F_{1,72} = 0.654$ ($p = 0.421$)	$F_{1,72} = 18.77$ ($p < 0.001$)	$F_{1,72} = 1.83$ ($p = 0.180$)	$F_{1,72} = 33.28$ ($p < 0.001$)
Species x Sex	$F_{1,72} = 14.68$ ($p < 0.001$)	$F_{1,72} = 14.56$ ($p < 0.001$)	$F_{1,72} = 0.215$ ($p = 0.644$)	$F_{1,72} = 12.57$ ($p = 0.001$)	$F_{1,72} = 10.04$ ($p = 0.002$)	$F_{1,72} = 14.37$ ($p < 0.001$)
Sex x Geography	$F_{1,72} = 0.387$ ($p = 0.536$)	$F_{1,72} = 2.65$ ($p = 0.108$)	$F_{1,72} = 22.62$ ($p < 0.001$)	$F_{1,72} = 4.14$ ($p = 0.046$)	$F_{1,72} = 8.97$ ($p = 0.004$)	$F_{1,72} = 1.54$ ($p = 0.218$)
Sex x Geography	$F_{1,72} = 3.21$ ($p = 0.077$)	$F_{1,72} = 5.34$ ($p = 0.024$)	$F_{1,72} = 13.10$ ($p = 0.001$)	$F_{1,72} = 0.001$ ($p = 0.977$)	$F_{1,72} = 2.44$ ($p = 0.123$)	$F_{1,72} = 2.47$ ($p = 0.121$
Species x Sex x Geography	$F_{1,72} = 0.209$ ($p = 0.649$)	$F_{1,72} = 5.13$ ($p = 0.027$)	$F_{1,72} = 0.941$ ($p = 0.335$)	$F_{1,72} = 0.107$ ($p = 0.744$)	$F_{1,72} = 0.004$ ($p = 0.949$)	$F_{1,72} = 5.44$ ($p = 0.022$)

Table 3: Correlations (Pearson correlation coefficients) between relative peak areas of the 24 cuticular hydrocarbons used in principal components 1-6 of *C. cobaltinus* and *C. auratus* allopatric and sympatric populations. Asterisks indicate significant contributors to each principal component (see Methods).

Compound	PC1	PC2	PC3	PC4	PC5	PC6
n-docosane	-0.078	0.783*	-0.025	-0.145	-0.169	0.401
2-methyldocosane	0.224	0.898*	0.133	0.123	0.021	0.000
9-tricosene	0.306	0.841*	0.228	0.258	0.005	-0.100
n-tricosane	0.181	0.917*	0.188	-0.120	0.064	-0.007
n-tetracosane	-0.206	0.529	0.094	-0.164	0.038	0.572
2-methyltetracosane	0.205	0.310	0.791*	0.160	0.003	0.223
9-pentacosene	0.168	0.169	0.726*	0.518	0.018	-0.048
n-pentacosane	0.025	0.124	0.907*	0.013	0.079	0.098
2-methylhexacosane	-0.058	-0.016	0.241	-0.002	0.064	0.820*
n-heptacosane	-0.135	-0.083	0.495	0.231	0.642*	0.269
2-methylheptacosane	-0.119	0.230	-0.243	0.406	-0.233	0.435
2-methyloctacosane	0.418	0.119	-0.030	0.359	0.718*	-0.135
9-nonacosene	0.083	0.006	0.393	0.843*	0.123	-0.042
n-nonacosane	0.207	-0.043	0.012	0.013	0.877*	0.013
2-methylnonacosane	0.573	0.277	-0.148	0.504	0.194	0.071
n-triacontane	0.862*	0.144	-0.112	0.147	0.038	-0.092
2-methyltriacontane	0.753*	0.210	-0.059	0.326	0.408	-0.173
9-hentriacontene	0.632*	0.206	0.300	0.550	0.077	-0.013
n-hentriacontane	0.798*	-0.082	0.193	0.101	0.408	-0.170
13-methylhentriacontane 15-methylhentriacontane	0.237	-0.268	0.118	0.775*	0.274	-0.026
n-dotriacontane	0.799*	-0.020	0.112	0.042	-0.019	-0.087
2-methyldotricacontane	0.830*	0.156	0.074	-0.054	0.062	0.165
n-tritriacontane	0.650*	0.132	0.378	0.065	0.100	-0.232

Table 4: Identification of GC peaks used in principal components analysis, and their mean relative peak areas (mean % of total combined area (S.E.)) in *C. cobaltinus*, *C. auratus*, and hybrid males and females.

			Relative Peak Areas (Mean % of total combined area (S.E.))									
			Allopatric				Sympatric					
Peak	Compound	Retention Time, Min.	*cobaltinus* males	*cobaltinus* females	*auratus* males	*auratus* females	*cobaltinus* males	*cobaltinus* females	*auratus* males	*auratus* females	*hybrid* males	*hybrid* females
1	n-docosane	10.944	0.76 (0.07)	0.38 (0.12)	0.59 (0.16)	0.35 (0.24)	0.09 (0.02)	0.16 (0.08)	0.04 (0.01)	0.00 (0.00)	0.05 (0.01)	0.01 (0.00)
2	2-methyldocosane	11.471	1.92 (0.35)	—	0.45 (0.18)	0.19 (0.16)	0.55 (0.13)	0.00 (0.00)	0.55 (0.10)	0.08 (0.02)	0.60 (0.08)	0.05 (0.02)
3	9-tricosene	11.596	8.21 (1.37)	0.25 (0.10)	2.48 (0.81)	0.45 (0.36)	3.47 (0.70)	0.16 (0.03)	4.13 (0.80)	0.55 (0.12)	3.39 (0.52)	0.33 (0.07)
4	n-tricosane	11.790	2.89 (0.37)	0.22 (0.09)	0.64 (0.19)	0.59 (0.37)	0.85 (0.14)	0.14 (0.06)	0.42 (0.05)	0.24 (0.03)	0.62 (0.08)	0.19 (0.04)
5	n-tetracosane	12.612	1.11 (0.05)	1.21 (0.10)	1.52 (0.17)	1.52 (0.43)	0.29 (0.04)	0.47 (0.10)	0.33 (0.03)	0.25 (0.01)	0.26 (0.02)	0.22 (0.01)
6	2-methyltetracosane	13.095	1.33 (0.19)	1.99 (0.83)	0.35 (0.11)	0.64 (0.54)	1.00 (0.20)	0.42 (0.08)	1.33 (0.52)	0.36 (0.07)	0.55 (0.06)	0.46 (0.08)
7	9-pentacosene	13.215	0.93 (0.10)	1.13 (0.29)	0.40 (0.15)	0.35 (0.27)	1.12 (0.39)	0.30 (0.15)	3.02 (1.15)	0.80 (0.24)	1.04 (0.21)	0.92 (0.21)
8	n-pentacosane	13.390	3.42 (0.36)	5.29 (1.66)	7.50 (6.13)	2.89 (0.86)	1.98 (0.58)	1.40 (0.22)	2.73 (0.75)	2.12 (0.17)	1.36 (0.27)	2.02 (0.30)
9	2-methylhexacosane	14.606	1.23 (0.26)	2.89 (0.54)	0.92 (0.37)	1.93 (0.82)	0.37 (0.06)	0.47 (0.05)	0.31 (0.08)	0.37 (0.03)	0.39 (0.07)	0.62 (0.08)
10	n-heptacosane	14.882	2.70 (0.24)	3.96 (0.34)	2.43 (0.44)	5.20 (0.70)	1.84 (0.33)	1.82 (0.07)	1.79 (0.18)	3.10 (0.17)	1.40 (0.14)	2.39 (0.18)
11	2-methylheptacosane	15.331	0.57 (0.09)	0.46 (0.14)	1.46 (0.56)	1.42 (0.84)	0.28 (0.04)	0.31 (0.03)	0.25 (0.02)	0.44 (0.02)	0.27 (0.02)	0.43 (0.02)
12	n-octacosane	15.617	1.95 (0.15)	2.22 (0.18)	3.70 (1.36)	3.42 (0.60)	1.36 (0.19)	1.90 (0.16)	1.01 (0.06)	1.74 (0.13)	1.00 (0.07)	1.43 (0.05)
13	2-methyloctacosane	16.110	21.11 (1.18)	20.35 (1.40)	18.15 (1.99)	29.85 (2.52)	21.00 (1.97)	24.93 (1.38)	18.33 (0.99)	31.80 (1.81)	21.48 (1.01)	30.46 (1.23)
14	9-nonacosene	16.261	1.48 (0.19)	2.79 (0.15)	3.61 (0.42)	2.91 (0.38)	1.28 (0.22)	2.72 (1.36)	5.12 (0.77)	3.39 (0.43)	1.80 (0.26)	2.50 (0.34)
15	n-nonacosane	16.431	11.51 (0.75)	19.02 (1.04)	8.71 (0.99)	20.14 (1.36)	9.85 (1.21)	13.49 (1.59)	8.51 (0.55)	17.47 (0.70)	9.22 (0.65)	15.06 (0.97)
16	2-methylnonacosane	16.993	1.24 (0.17)	0.99 (0.15)	2.06 (0.52)	1.03 (0.16)	1.07 (0.13)	1.07 (0.03)	1.17 (0.07)	1.22 (0.05)	1.27 (0.06)	1.21 (0.04)
17	n-triacontane	17.360	1.90 (0.19)	1.66 (0.08)	2.35 (0.52)	1.21 (0.05)	2.43 (0.21)	2.64 (0.23)	1.42 (0.16)	1.33 (0.09)	1.63 (0.08)	1.42 (0.05)
18	2-methyltriacontane	18.006	24.64 (1.26)	17.27 (1.25)	29.05 (3.73)	17.82 (1.86)	34.62 (2.51)	29.81 (0.98)	35.42 (3.02)	24.77 (0.85)	39.60 (1.34)	27.19 (1.21)
19	9-hentriacontene	18.222	2.59 (0.31)	3.57 (0.37)	3.14 (0.33)	0.78 (0.18)	4.22 (0.78)	3.72 (0.41)	5.73 (0.90)	1.89 (0.19)	4.91 (0.47)	3.40 (0.54)
20	n-hentriacontane	18.437	4.30 (0.42)	5.14 (0.31)	3.33 (0.38)	3.91 (0.44)	6.59 (0.65)	6.84 (0.43)	3.91 (0.28)	4.70 (0.18)	4.15 (0.47)	4.87 (0.30)
21	13-methylhentriacontane 15-methylhentriacontane	18.762	0.38 (0.42)	1.44 (0.31)	1.19 (0.28)	0.87 (0.18)	0.47 (0.09)	1.85 (0.18)	1.72 (0.15)	1.94 (0.14)	1.43 (0.28)	2.84 (0.25)
22	n-dotriacontane	19.707	0.43 (0.12)	0.50 (0.19)	0.09 (0.05)	— —	0.45 (0.07)	0.74 (0.16)	0.46 (0.08)	0.26 (0.03)	0.43 (0.02)	0.26 (0.02)
23	2-methyldotriacontane	20.629	1.61 (0.14)	2.12 (0.37)	2.36 (0.48)	0.62 (0.26)	2.31 (0.29)	1.83 (0.16)	1.56 (0.34)	0.62 (0.05)	2.28 (0.23)	1.00 (0.11)
24	n-tritriacontane	21.219	0.73 (0.24)	0.29 (0.09)	0.07 (0.05)	0.03 (0.03)	0.62 (0.13)	0.54 (0.14)	0.52 (0.07)	0.47 (0.02)	0.61 (0.06)	0.52 (0.06)

Table 5: MANOVA of PCs for *C. cobaltinus* and *C. auratus* parentals and hybrids.

Source	F-value	p-value
Species	$F_{12,98} = 7.79$	$p < 0.001$
Sex	$F_{6,49} = 38.9$	$p < 0.001$
Species x Sex	$F_{12,98} = 3.26$	$p < 0.001$

Table 6: ANOVA values for *C. cobaltinus* and *C. auratus* parentals and hybrids. F- and p- values indicate which source influenced each PC.

Source	PC1	PC2	PC3	PC4	PC5	PC6
Species	$F_{2,54} = 0.032$ ($p = 0.968$)	$F_{2,54} = 2.53$ ($p = 0.089$)	$F_{2,54} = 1.80$ ($p = 0.175$)	$F_{2,54} = 6.13$ ($p = 0.004$)	$F_{2,54} = 18.60$ ($p < 0.001$)	$F_{2,54} = 6.71$ ($p = 0.002$)
Sex	$F_{1,54} = 50.07$ ($p < 0.001$)	$F_{1,54} = 4.93$ ($p = 0.031$)	$F_{1,54} = 17.10$ ($p < 0.001$)	$F_{1,54} = 1.84$ ($p = 0.180$)	$F_{1,54} = 0.15$ ($p = 0.703$)	$F_{1,54} = 0.33$ ($p = 0.569$)
Species x Sex	$F_{2,54} = 0.42$ ($p = 0.662$)	$F_{2,54} = 1.86$ ($p = 0.166$)	$F_{2,54} = 1.32$ ($p = 0.276$)	$F_{2,54} = 1.26$ ($p = 0.291$)	$F_{2,54} = 11.90$ ($p < 0.001$)	$F_{2,54} = 4.77$ ($p = 0.012$)

Table 7: Correlations (Pearson correlation coefficients) between relative peak areas of the 24 cuticular hydrocarbons used in principal components 1-6 of *C. cobaltinus* and *C. auratus* parentals and hybrids. Asterisks indicate significant contributors to each principal component (see Methods).

Compound	PC1	PC2	PC3	PC4	PC5	PC6
n-docosane	0.550	-0.152	-0.025	-0.036	-0.095	0.724*
2-methyldocosane	0.835*	0.086	0.286	0.194	0.159	0.207
9-tricosene	0.886*	0.253	0.212	0.020	0.171	0.083
n-tricosane	0.842*	0.168	0.259	0.186	-0.151	0.145
n-tetracosane	0.373	0.392	0.287	0.165	0.107	0.639*
2-methyltetracosane	0.340	0.860*	0.122	-0.020	-0.001	0.220
9-pentacosene	0.294	0.852*	0.003	-0.029	0.302	0.097
n-pentacosane	-0.029	0.923*	0.119	0.098	0.023	-0.144
2-methylhexacosane	-0.134	0.606	0.164	0.602	-0.064	0.289
n-heptacosane	-0.138	0.729*	-0.056	0.344	0.147	-0.405
2-methylheptacosane	0.095	0.185	0.147	0.902*	0.157	0.081
2-methyloctacosane	0.068	0.034	0.300	0.882*	0.217	-0.067
9-nonacosene	0.232	0.565	-0.048	0.010	0.722*	-0.034
n-nonacosane	0.335	-0.049	-0.224	0.603	-0.186	-0.385
2-methylnonacosane	0.529	-0.047	0.503	0.511	0.329	0.078
n-triacontane	0.656*	-0.182	0.479	-0.177	-0.212	0.303
2-methyltriacontane	0.581	-0.040	0.692*	0.289	0.218	0.042
9-hentriacontene	0.684*	0.308	0.323	0.034	0.384	0.108
n-hentriacontane	0.221	0.194	0.623*	0.124	-0.302	0.043
13-methylhentriacontane 15-methylhentriacontane	0.047	0.091	-0.156	0.442	0.749*	0.020
n-dotriacontane	0.316	-0.223	0.612*	-0.12	0.117	0.452
2-methyldotriacontane	0.403	-0.024	0.833*	0.122	-0.085	0.176
n-dimethyltritriacontane	0.102	0.309	0.821*	0.154	-0.014	0.069

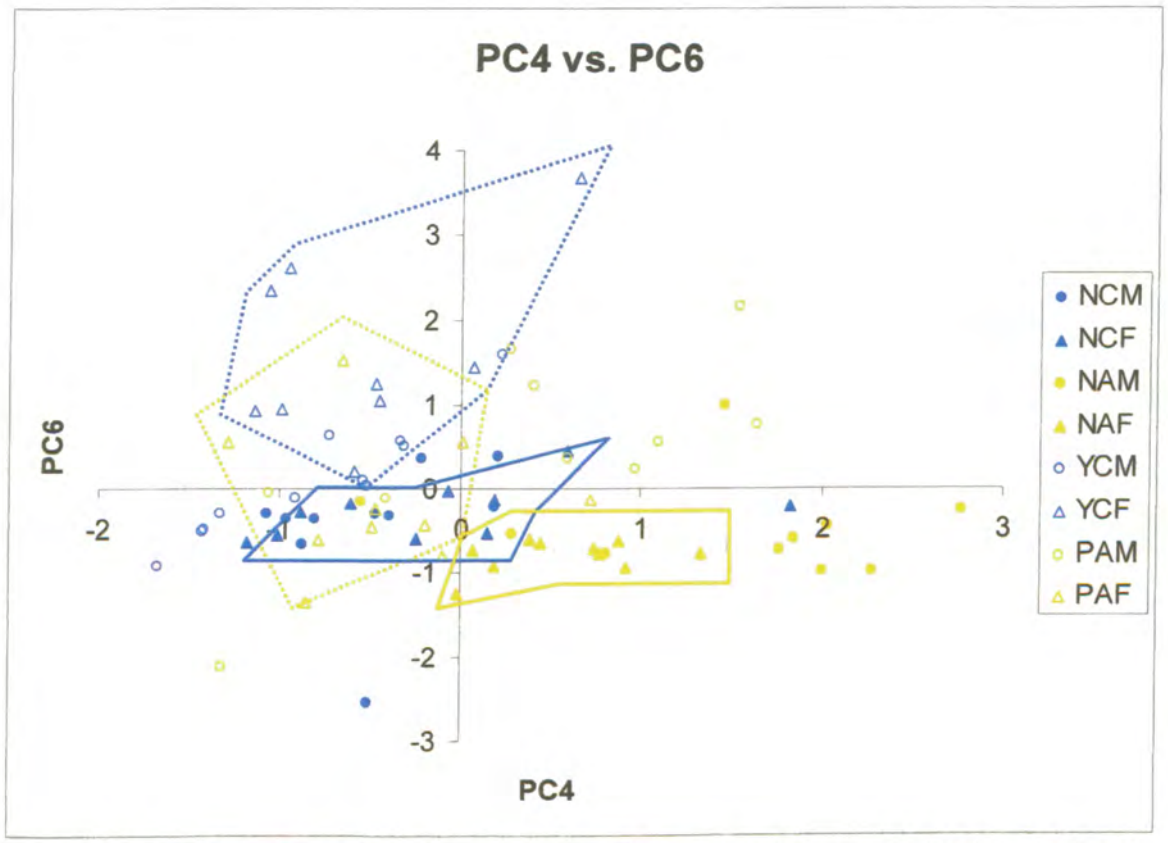


Figure 1: Representative principal components plot, with PC scores for PC4 and PC 6 for allopatric *C. cobaltinus* and *C. auratus* males (YCM and PAM, repectively) and females (YCF and PAF, respectively) and sympatric *C. cobaltinus* and *C. auratus* males (NCM and NAM, respectively) and females (NCF and NCM, respectively). Outlining of the four groups of females indicates more overlap between allopatric females than sympatric females, consistent with reproductive character displacement.

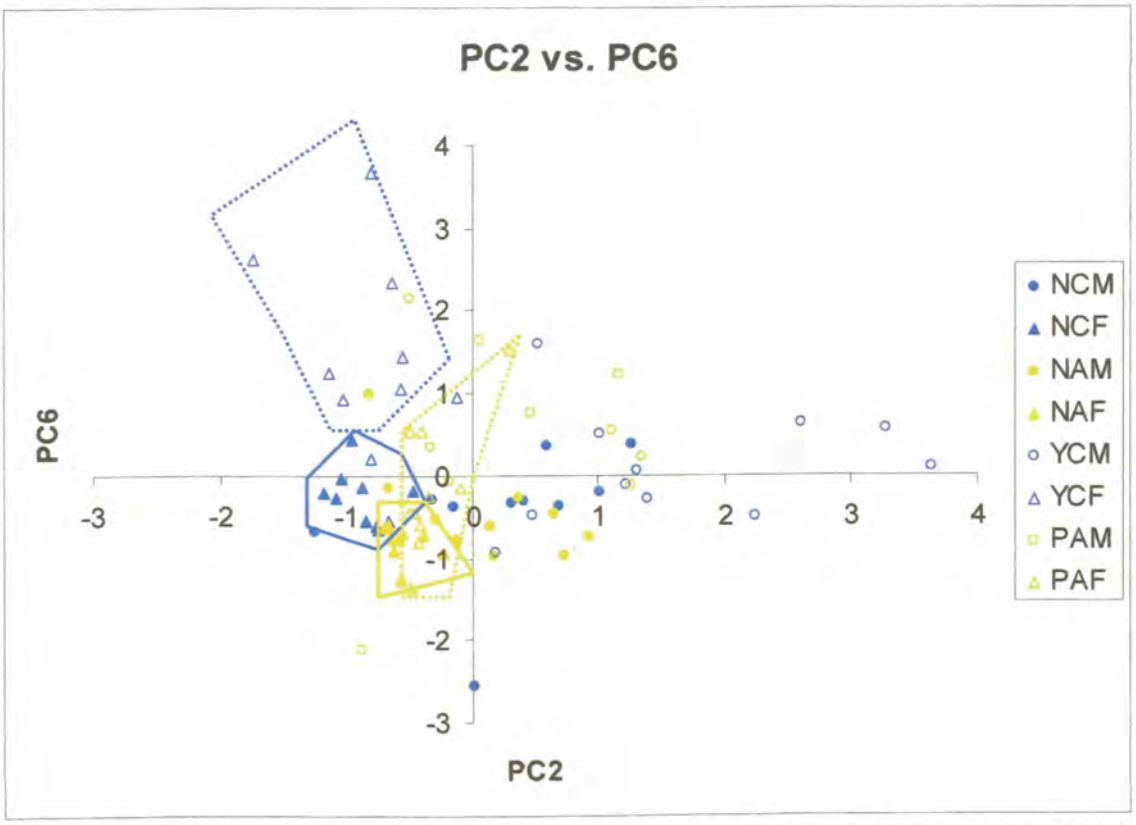


Figure 2: Representative principal components plot, with PC scores for PC2 and PC6 for allopatric *C. cobaltinus* and *C. auratus* males (YCM and PAM, repectively) and females (YCF and PAF, respectively) and sympatric *C. cobaltinus* and *C. auratus* males (NCM and NAM, respectively) and females (NCF and NCM, respectively). Outlining indicates no Greater overlap for allopatric females than for sympatric females, inconsistent with Reproductive character displacement.

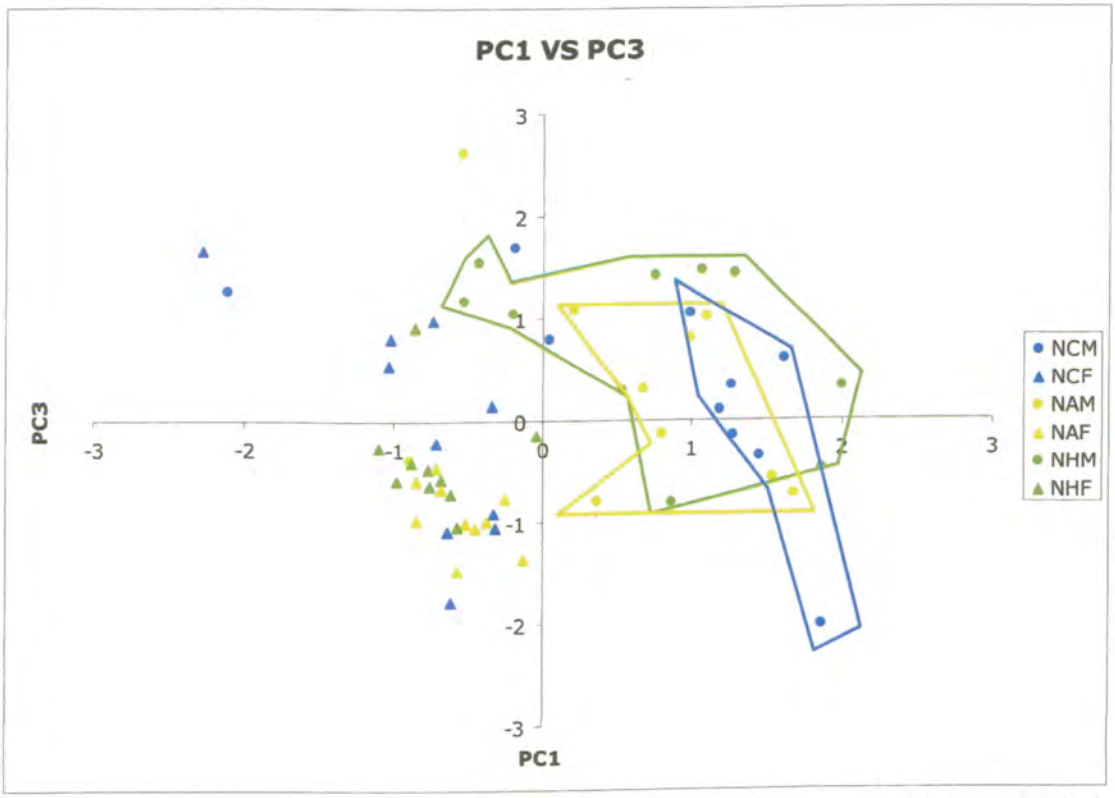


Figure 3: Representative principal components plot, with PC scores for PC1 and PC3 for sympatric *C. cobaltinus* males (NCM) and females (NCF); *C. auratus* males (NAM) and females (NAF); and hybrid males (NHM) and females (NHF). Outlining shows more overlap of male hybrids with male *C. auratus* than with male *C. cobaltinus*.

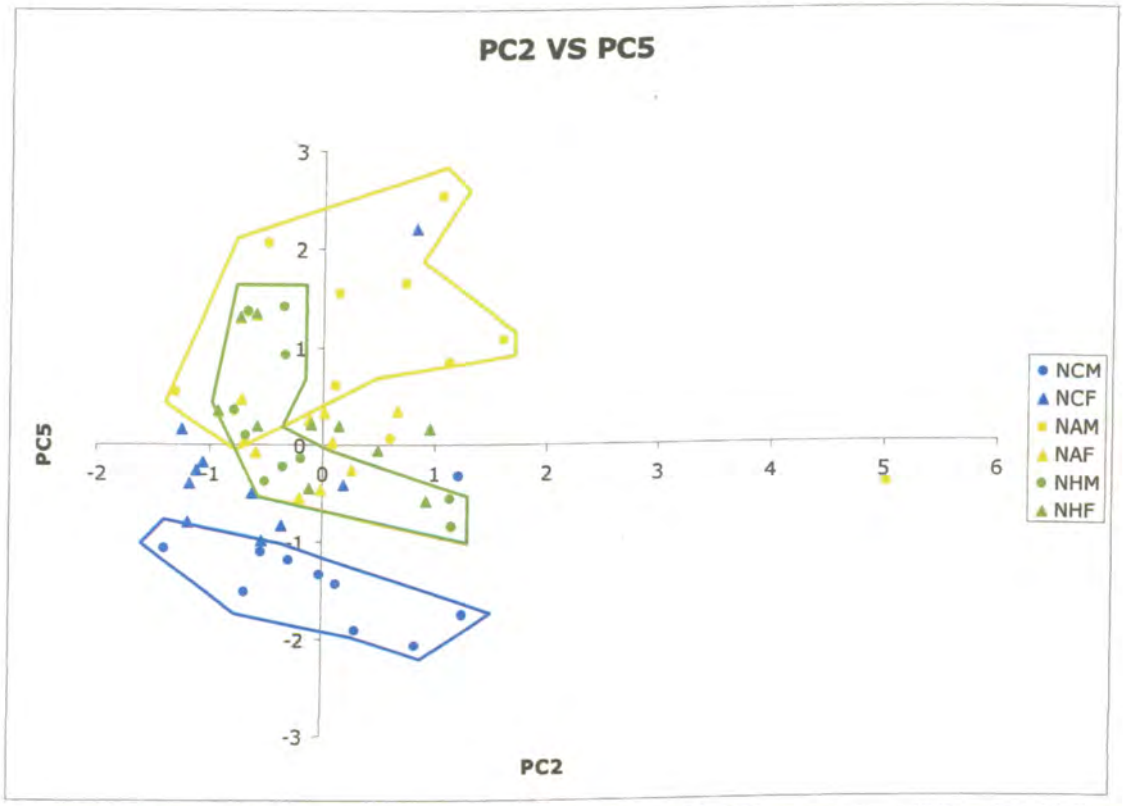


Figure 4: Representative principal components plot, with PC scores for PC2 and PC5 for sympatric *C. cobaltinus* males (NCM) and females (NCF); *C. auratus* males (NAM) and females (NAF); and hybrid males (NHM) and females (NHF). Outlining shows more overlap of male hybrids with male *C. auratus* than with male *C. cobaltinus*.

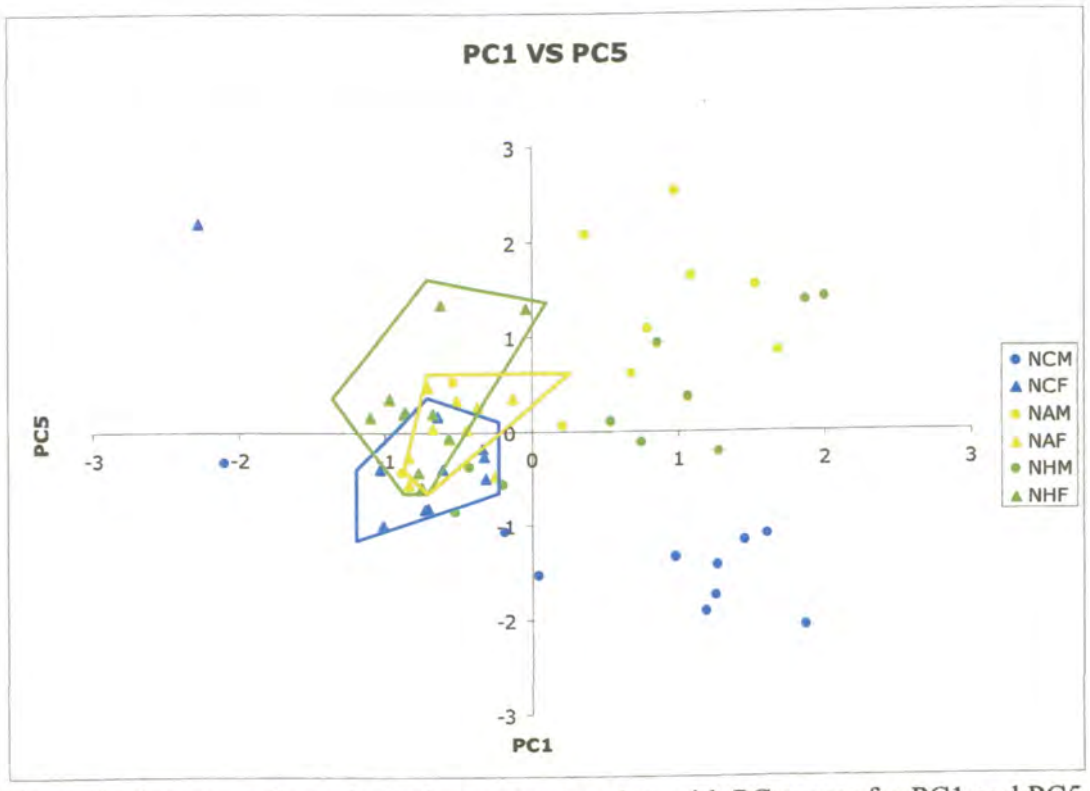


Figure 5: Representative principal components plot, with PC scores for PC1 and PC5 for sympatric *C. cobaltinus* males (NCM) and females (NCF); *C. auratus* males (NAM) and females (NAF); and hybrid males (NHM) and females (NHF). Outlining shows more overlap of female hybrids with female *C. auratus* than with female *C. cobaltinus*.

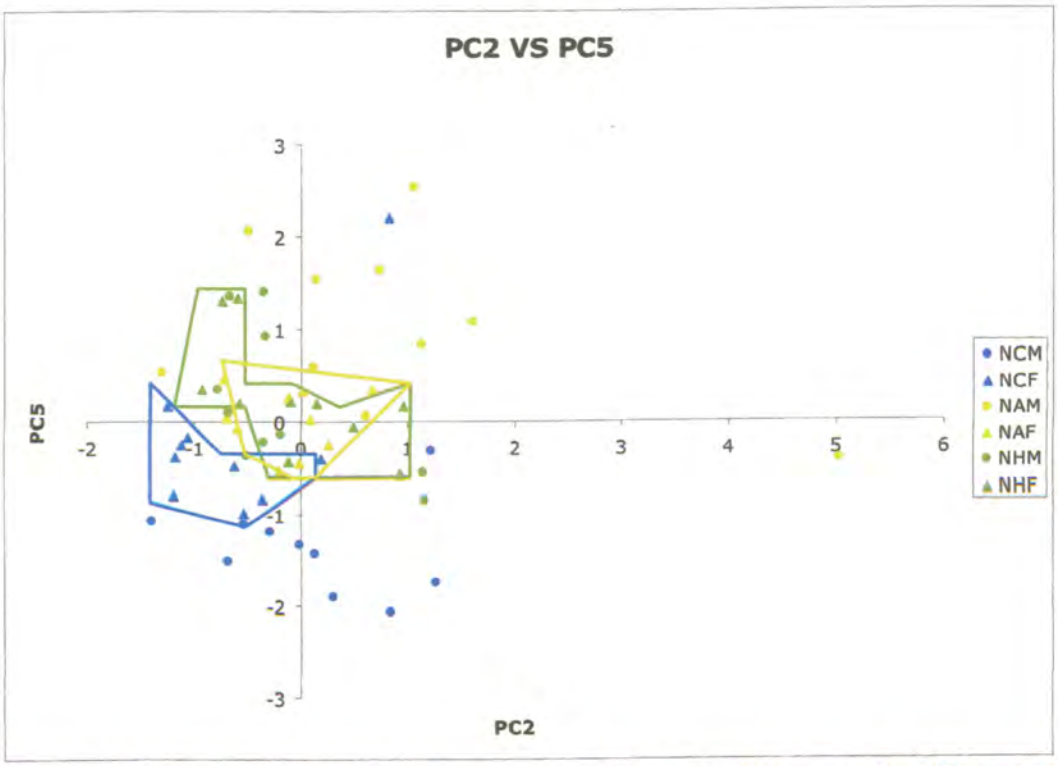


Figure 6: Representative principal components plot, with PC scores for PC2 and PC5 for sympatric *C. cobaltinus* males (NCM) and females (NCF); *C. auratus* males (NAM) and females (NAF); and hybrid males (NHM) and females (NHF). Outlining shows more overlap of female hybrids with female *C. auratus* than with female *C. cobaltinus*.

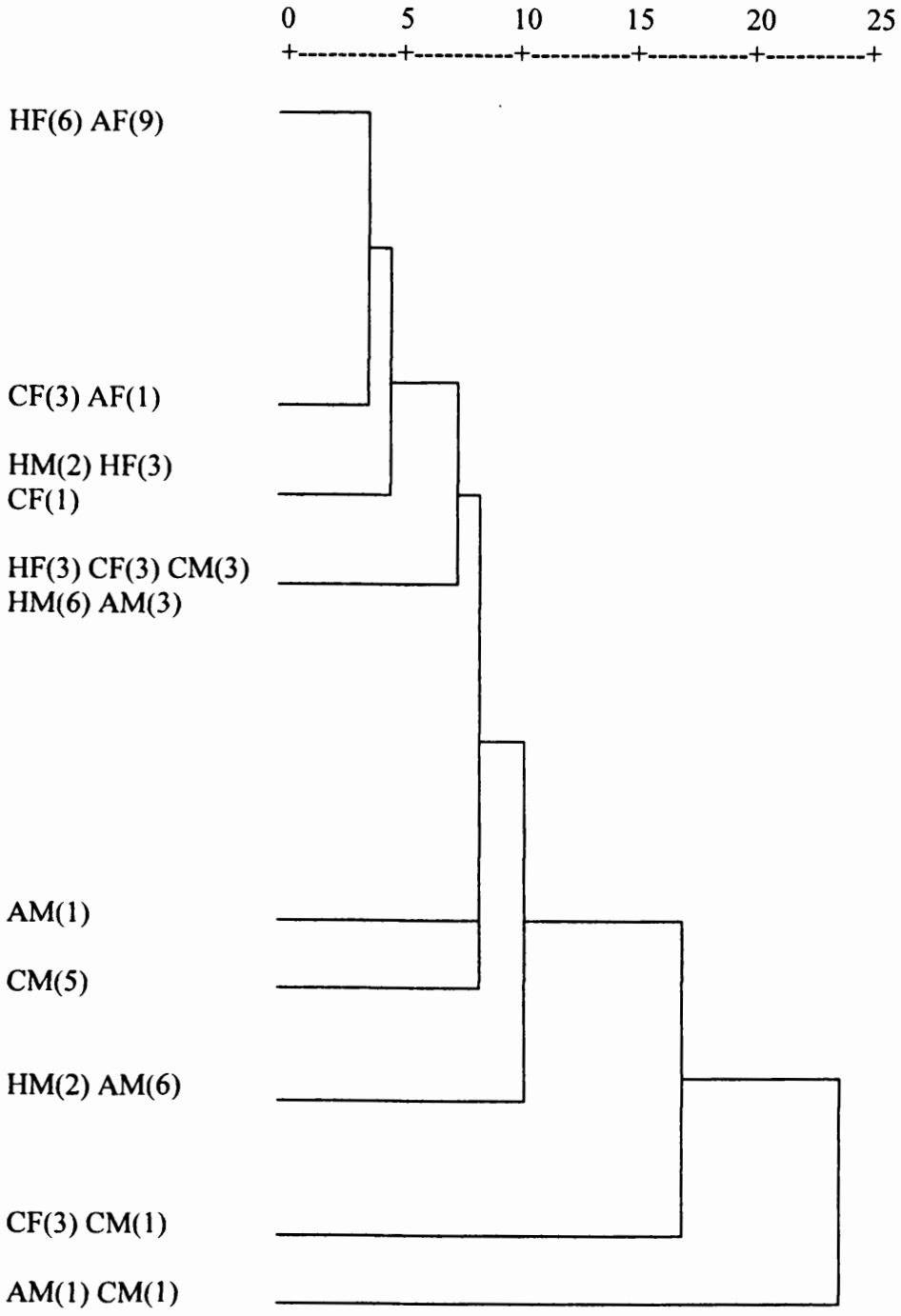
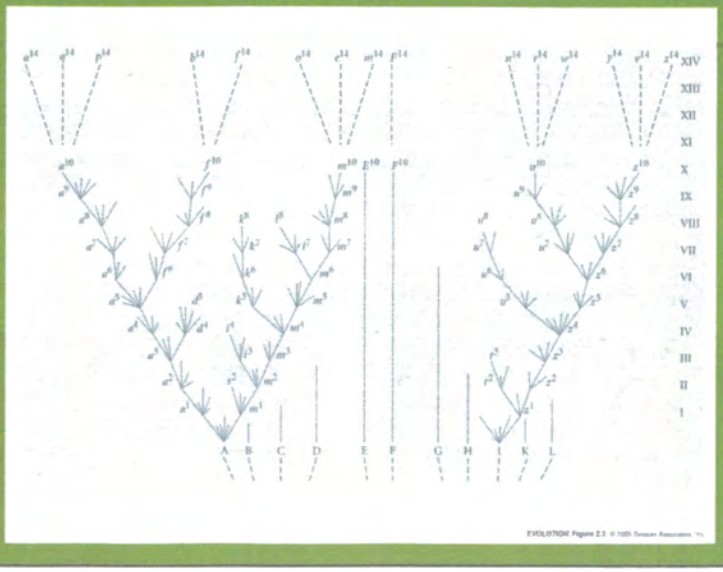


Figure 7: Cluster analysis of *C. cobaltinus* males (CM) and females (CF); *C. auratus* males (AM) and females (AF); and hybrid males (HM) and females (HF). Both male and female hybrids tend to cluster with *C. auratus* more than they cluster with *C. cobaltinus*.

Reproductive Character Displacement and X-linkage of Cuticular Hydrocarbons in *Chrysochus* Beetles

Hallie Kerins
Biology Department, Western Washington University

Since Darwin...



Speciation Research

- Focused on reproductive isolation with three lines of research:
  1. Evolutionary process of behavioral isolation
  2. Mechanistic basis for behavioral isolation
  3. The underlying genetic changes of reproductive isolation

1. Evolutionary process of behavioral isolation

- Biological species concept
  - = organisms are considered the same species if they are potentially capable of interbreeding and producing fertile offspring.

1. Evolutionary process of behavioral isolation

Reinforcement Theory
(Dobzhansky 1940)

= process by which natural selection against hybridization increases reproductive isolation between two closely related species in areas of secondary contact.

2. Mechanistic basis for behavioral isolation

- Wide range of mechanisms
- Chemical signaling system often related to reproductive isolation in insects
- Cuticular hydrocarbons

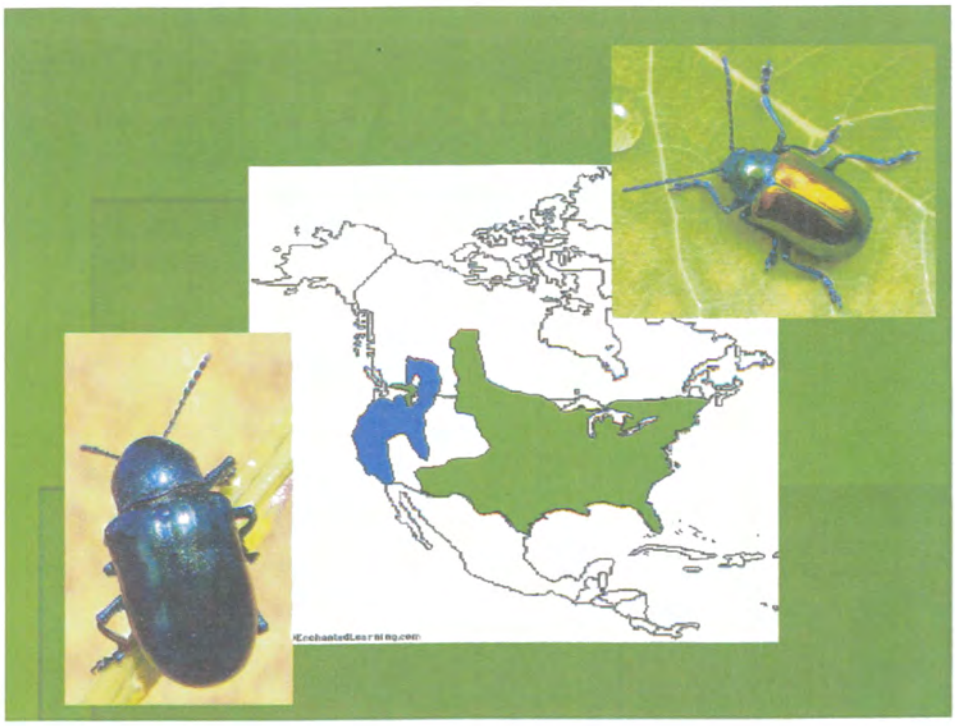
3. The underlying genetic changes of reproductive isolation

- Typical patterns:
  - Only a few genes involved in speciation
  - Often X-linked (maternally inherited)

The *Chrysochus* system

Chrysochus auratus

Chrysochus cobaltinus



Chrysochus mating system

- Mate daily throughout 6-8 week lifespan
- Hybridize frequently: 10-15% of individuals are hybrids
- Hybrids effectively sterile
- Parentals show positive assortative mating



Hypotheses

1. Reproductive character displacement can be seen in CHCs
2. CHC profiles are X-linked

In general...

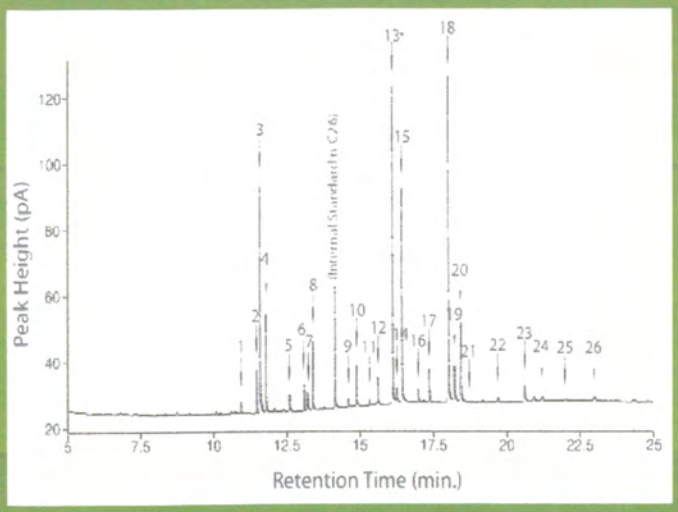
- To test the hypothesis #1
  - → Compared males and females of allopatric and sympatric parentals
  - →See whether or not CHC profiles are more divergent in sympatry
- To test hypothesis #2
  - → Compared males and females of parentals and of hybrids
  - →See whether or not hybrids had CHC that hinted at a pattern of inheritance

Hypothesis #1: Reproductive Character Displacement of CHC Profiles

- Compared:
  - 40 sympatric parentals: 10 males and 10 females from each *C. cobaltinus* and *C. auratus*
  - 40 allopatric parentals: 11 male and 9 female *C. cobaltinus* and 10 male and 10 female *C. auratus*

What we did...

- Obtained hydrocarbon extracts
- GC analysis



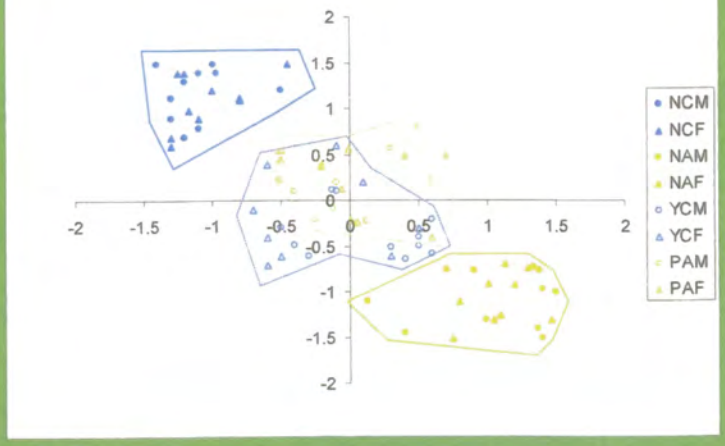
- Aligned peaks across individuals using internal standard
- Relative peak areas
- Principal components analysis

Hypothesis #1: Reproductive Character Displacement of CHC Profiles

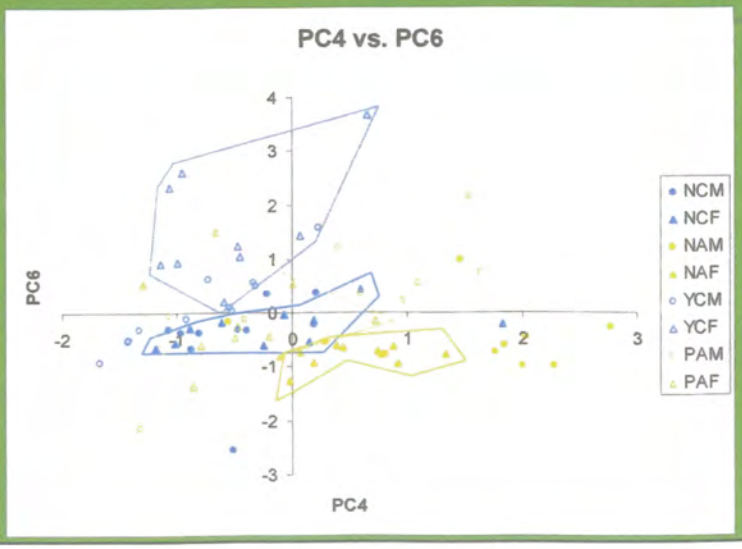
- Principal component analysis resulted in 6 PCs
- Together explained 80.47% of the total variance:
  - PC1: 21.9%
  - PC2: 16.4%
  - PC3: 12.8%
  - PC4: 12.2%
  - PC5: 9.9%
  - PC6: 7.3%

Hypothesis #1: Reproductive Character Displacement of CHC Profiles

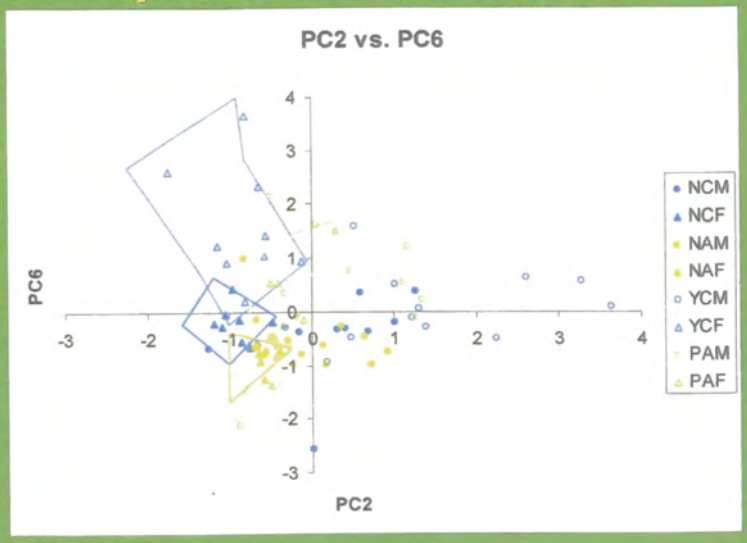
Expected:



Hypothesis #1: Reproductive Character Displacement of CHC Profiles



Hypothesis #1: Reproductive Character Displacement of CHC Profiles



Hypothesis #1: Reproductive Character Displacement of CHC Profiles

- Conclusions:
  - We found the expected pattern for some PCs, but the pattern was far from universal
  - At present, we cannot tell whether cues for mating are more or less divergent between allopatric and sympatric populations.

Hypothesis #2: Are CHC profiles X-linked?

- Compared:
  - 40 sympatric parentals: 10 males and 10 females from each *C. cobaltinus* and *C. auratus*
  - 20 hybrid individuals

Hypothesis #2: Are CHC profiles X-linked?

- Principal component analysis resulted in 6 PCs
- Together explained 85.56% of the total variance:
  - PC1: 21.3%
  - PC2: 18.1%
  - PC3: 16.2%
  - PC4: 13.9%
  - PC5: 8.1%
  - PC6: 8.0%

Hypothesis #2: Are CHC profiles X-linked?

- 90% hybrids are result of mating between *C. auratus* male and *C. cobaltinus* female

XX

XY

XX

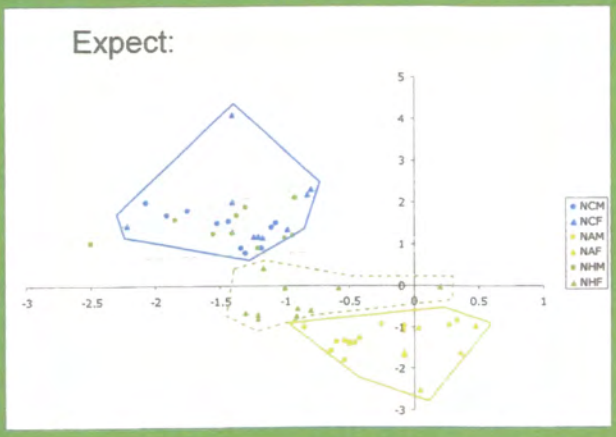
XY

Hypothesis #2: Are CHC profiles X-linked?

- If CHCs are maternally inherited (X-linked)
  - →Males would exhibit CHCs similar to *C. cobaltinus*
  - →Felmales would exhibit CHCs intermediate between *C. cobaltinus* and *C. auratus*
- If CHCs autosomally inherited
  - →both sexes would exhibit CHCs intermediate between *C. cobaltinus* and *C. auratus*

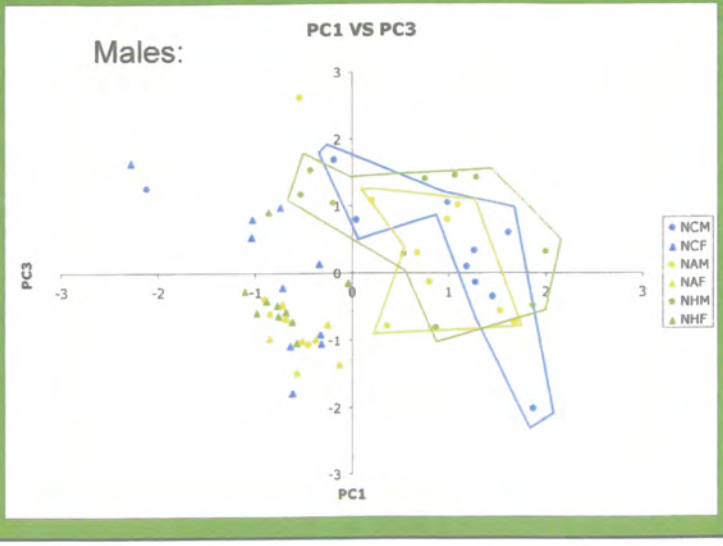
Hypothesis #2: Are CHC profiles X-linked?

Expect:



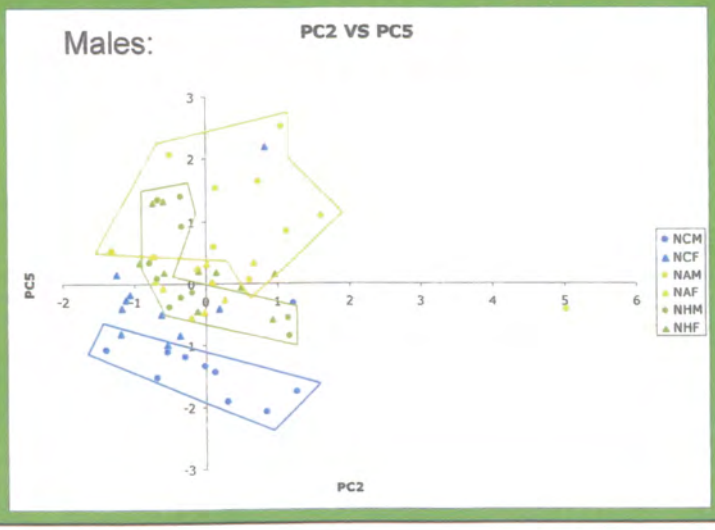
Hypothesis #2: Are CHC profiles X-linked?

Males:



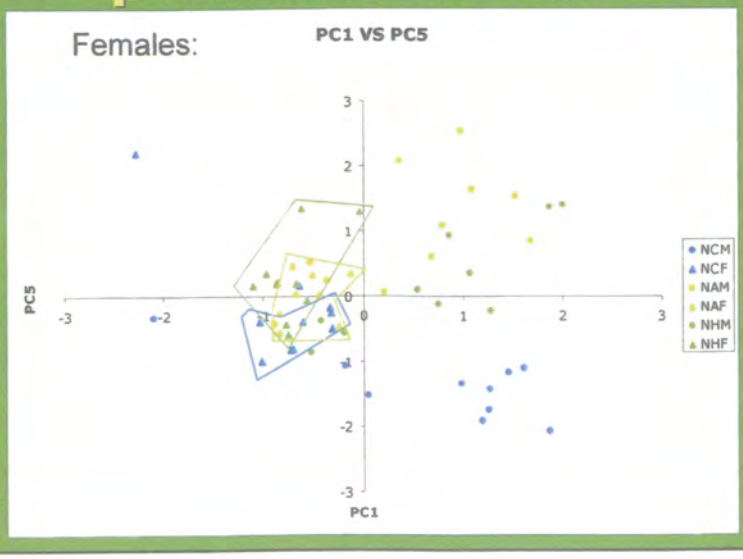
Hypothesis #2: Are CHC profiles X-linked?

Males:

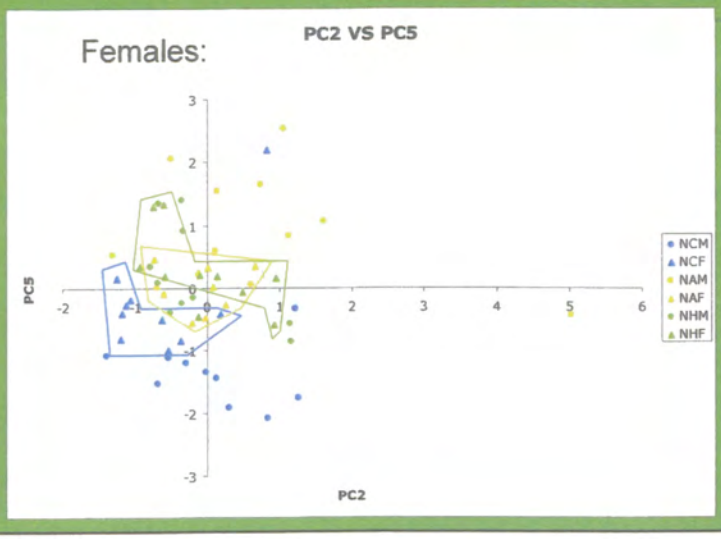


Hypothesis #2: Are CHC profiles X-linked?

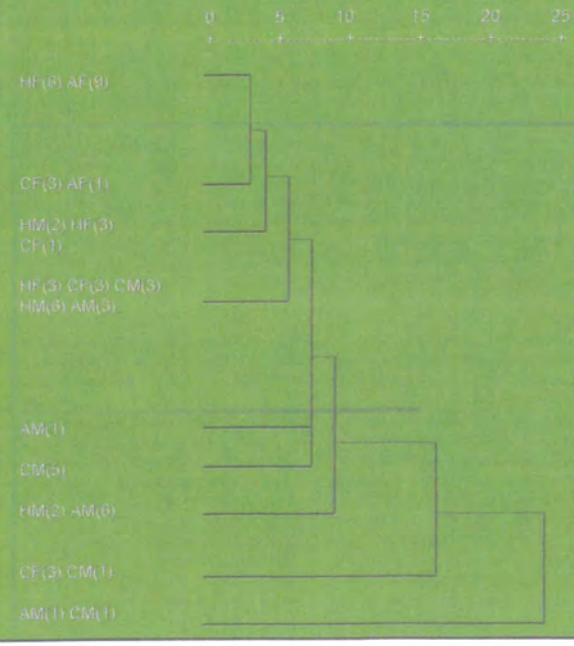
Females:



Hypothesis #2: Are CHC profiles X-linked?



Hypothesis #2: Are CHC profiles X-linked?



Hypothesis #2: Are CHC profiles X-linked?

- Conclusions:
  - Expected pattern is not seen in our results
  - Both PC plots and cluster analysis show hybrids clustering with *C. auratus* more than *C. cobaltinus*.

Future Research

1. Reproductive Character Displacement of CHC Profiles
   - Determine which peaks actually influence mate choice to see if PC influenced by those peaks are more divergent
2. Are CHC profiles X-linked?
   - More carfully controlled crosses
   - Quantitative genetics approach to determine the number of genes involved in CHCs

Acknowledgements

Special Thanks to:

Merrill Peterson
Susanne Dobler
Kirsten Monsen
Wittko Francke

Maggie Brassil
Kati Buckingham
Jabin Green
Jessica Mendoza
Erica Davis
Danielle Juarez

Funding:

National Science Foundation,
Bureau of Faculty Research, Western Washington Univ.,
Alexander von Humbolt Foundation, Germany