crustacean development and reproduction will eventually be useful in the mariculture of beneficial species, such as shrimp and lobster, and in the control of deleterious species, including predators, parasites, and fouling organisms among the crustaceans.

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Evolution of Male Pheromones in Moths: Reproductive Isolation Through Sexual Selection?

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Central to our understanding of the species concept is knowledge of the nature and evolution of reproductive isolating mechanisms. The once widely accepted model of Dobzhansky, which holds that isolation evolves through selection against hybrids of differentially adapted populations, is now largely rejected. This rejection is due to both theoretical difficulties and a paucity of examples of the predicted pattern of reproductive character displacement. From a survey of five families of Lepidoptera, entailing more than 800 species, evidence is given that male courtship pheromones have evolved within the context of sexual isolation as an adaptive response to mating mistakes between differentially adapted populations; however, distinct from the natural selection model of Dobzhansky, this report suggests the mechanism for change to be sexual selection.

THE MECHANISMS BY WHICH INDIviduals of two noncompatible populations are prevented from mating are among the most important characteristics of species, representing the cornerstone of the biological species concept (*1, 2*); elucidation of how such mechanisms arise is important to our complete understanding of the speciation process. Barriers to mating that arise due to incompatibilities in behavior are termed ethological isolating mechanisms and represent the most important group of isolating mechanisms in animals (*1*). Theories that have been proposed to explain the origin of ethological isolating mechanisms may be characterized as either adaptive or incidental. According to the former group, ethological isolation arises as a response to selection against mating with an individual whose genotype is adapted to a different niche (*3, 4*). Thus, the production of either infertile offspring or hybrids that are not optimally suited to either niche brings about an increased tendency to mate assortatively, that is, with individuals of similar genotype. Proponents of incidental models, on the other hand, argue that species-specific mating patterns are only an incidental by-product of the genetic divergence that occurs when two populations become isolated in time or space (*5, 6*). Accordingly, if the potential for interbreeding is reestablished, the two populations will already be sufficiently distinct in their mating behavior that individuals from different populations will not recognize each other as potential mates, and interpopulational matings will be rare. Within each of these categories, differences also arise as to whether the vehicle for change in mating patterns is natural selection (*3, 5*) or sexual selection (*4, 6*). Here we present evidence from five families of Lepidoptera that male courtship pheromones in moths have evolved within the context of reproductive isolation through sexual selection.

The presence of male scent–emitting structures in Lepidoptera, represented by various brush organs, hair pencils, and wing folds, is usually associated with a courtship sequence that allows their presentation to the female before mating; the importance of male pheromones to mating success has been documented for a number of diverse groups (*7*). We examined the relation between the occurrence of male scent–disseminating structures and the probability of contact with closely related species to test the predictions of the adaptive response and incidental models for the origin of lepidopteran male pheromones within the context of reproductive isolation. Interspecific contact was indexed by the sharing of a common host plant by two members of the same genus, on the assumption that species that utilize the same host are at greater risk of making mating mistakes than those that do not. However, when seasonal or geographical information was available on adult activity, it was used as further evidence for the probability of interspecific contact. Thus, with regard to moth mating systems, the distinction between the predictions of the models is clear and testable. If male pheromone–emitting structures have arisen as an adaptive response to mating mistakes, then they should be preferentially found in spe-

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cies that share a host plant with closely related species, that is, in those that have a higher probability of mating mistakes. Conversely, if these structures have only arisen fortuitously, while two populations were separated, there should be no relation between their occurrence and host overlap with congenerics.

The relation between male structure and host overlap was examined in five families of moths (*8*), chosen because each contains a mixture of species with and species without male scent structures and because extensive records of host plants were available for these taxa. In spite of an inherent methodological bias against finding such a relation (*9*), chi-square analyses of male pheromone–emitting structures and host overlap (Table 1) show significant positive correlations in each of the five lepidopteran families examined. When all taxa are combined, 53% (208 out of 396) of the species sharing at least one host with a congeneric had male pheromonal structures, whereas these structures were found in only 28% (118 out of 419) of the species not sharing a host. On a finer level, two tribes of tortricids, the Cnephasiini and Tortricini, are distinguished from other tribes by the virtually complete absence of the male forewing costal fold, a pheromone-emitting structure in this family. Within these two tribes, only 26% (23 out of 90) of the species show host overlap, compared to 59% (69 out of 117) for the rest of the family, which includes both species with and species without male costal folds. Thus, the results are consistent with the predictions of an adaptive response model for the origin of male pheromones in these families, and the predictions of the incidental models are not supported.

A similar conclusion is reached by means of a different measure of interspecific contact potential, size of geographical range. On the assumption that mating errors would be more likely, on average, in those species with broad spatial distributions, the relation between male structure and geographical range was examined in three of the above lepidopteran families for which there are good geographical data. In the North American Phycitinae (*8*), species with male structures have ranges encompassing 6.2 ± 0.5 (mean ± SE, $n = 207$) American states or Canadian provinces compared to 4.0 ± 0.7 (mean ± SE, $n = 41$) for species without male structures ($t = 2.44$, $P < 0.01$). In the British Tortricidae, specific geographical distributions have been classified as either widespread or restricted local populations (*8*). In a survey of 340 species, we found that species with male costal folds are more likely to be described as widespread than species without these structures ($\chi^2 = 15.4$, $P < 0.001$). In the third group examined, the Japanese Yponomeutidae (*8*), no significant relation was determined for the occurrence of male coremata and breadth of distribution, as measured by the number of Japanese islands inhabited.

Table 1. Chi-square analysis of the relations between the presence of male scent–emitting structures and the sharing of a host plant with congeneric species; n is the number of species.

Familes and incidence of male scent organs*	Host-plant overlap		Statistical analysis
	+	–	
Phycitinae (Pyralidae)—Nearctic & Palearctic			$n = 175$
Present	71	63	$\chi^2 = 14.2$
Absent	8	33	$P = 0.0004$
Yponomeutidae—Japan			$n = 56$
Present	33	11	$\chi^2 = 10.3$
Absent	3	9	$P = 0.002$
Tortricidae—British Isles			$n = 305$
Present	39	21	$\chi^2 = 17.8$
Absent	86	159	$P = 0.0001$
Noctuidae—British Isles			$n = 252$
Present	55	20	$\chi^2 = 12.5$
Absent	87	90	$P = 0.0007$
Ethmiidae—New World			$n = 27$
Present	10	3	$\chi^2 = 6.3$
Absent	4	10	$P = 0.01$
Total			$n = 815$
Present	208	118	$\chi^2 = 50.4$
Absent	188	301	$P < 0.0001$

*Phycitinae, abdominal hair pencils; Yponomeutidae, abdominal coremata; Tortricidae, forewing costal fold; Noctuidae, abdominal brush organs; Ethmiidae, hindwing hair pencils.

The relations seen in two lepidopteran groups for which reproductive isolation has been closely examined are also consistent with the origin of male scent–disseminating structures through an adaptive response context. Members of the stored-product phycitine complex exhibit broad reproductive overlap in time, space, and female pheromonal chemistry (*10*), resulting in a high potential for cross-attraction. In these species, we find an elaborate courtship that showcases male pheromones (*11*). In the Sesiidae, just the opposite relation can be found. Measuring the relative contribution of various parameters of reproductive isolation in eight sesiid species, Greenfield and Karandinos (*12*) found that 93% of the species pairs achieved high reproductive isolation (overlap of <5%) due to a single parameter. The remaining overlap was eliminated by species partitioning of additional parameters. Thus, they concluded that reproductive isolation in this group is a long-range phenomenon that operates "before the male and female enter the courtship arena" (*12*, p. 403). Consistent with an adaptive response interpretation, male pheromone–disseminating structures are not apparent in these species, as evidenced by taxonomic studies and behavioral observations (*13*).

According to the adaptive response model of Dobzhansky (*3*), disruptive natural selection against hybrids brings about a behavioral or morphological divergence in sexual characters between two differently adapted populations after the reestablishment of sympatry. A differentiation of mating behavior in the zone of overlapping ranges (reproductive character displacement) is predicted since selection is operating only in the area of populational overlap, and not where the incipient species are allopatric. This hypothesis, once widely accepted, has recently been criticized (*2, 5, 6*) for a number of theoretical deficiencies and because very few cases of the predicted reproductive character displacement have been reported, in spite of many attempts to find them (*14*). According to opponents of Dobzhansky's model, the citing of a few cases of apparent reproductive character displacement is simply a case of sampling bias, and just as many examples showing the reverse relation can be found (*1*). Consideration of the difficulties of the reinforcement model has led to the expectation that reproductive isolation "will evolve . . . through the direct action of natural selection only under a rather restricted range of conditions" (*2*, p. 328).

The lack of examples of reproductive character displacement has been cited as evidence to support incidental models and has even led to a call for the rejection of the biological species concept (*5*). However, Fisher (*4*) also invoked the adaptive response context in a model for which sexual selection rather than natural selection acts as the vehicle for differentiation in mating patterns. Fisher (*4*) suggested that the avoidance of interpopulational matings that produce offspring of lower fitness may provide the initial genetic advantage that he considered necessary for runaway sexual selection to commence. Females with a mating preference that increases assortative mating will be at a reproductive advantage, since they will avoid producing hybrid offspring of reduced fitness, and males possessing the character that females prefer will obtain more matings. Assuming a significant character heritability, females will benefit further by mating with these males since their sons will possess the character and thus have a greater probability of mating in the next generation.

The important distinction between this model and Dobzhansky's (*3*) is that, because of the sexual selection mechanism, the spatial pattern of reproductive character displacement between sympatric and allopatric populations is not predicted. Rather, the new male character and associated female preference would start in the area of reproductive overlap, but on account of the runaway Fisherian process, would continue into the areas of allopatry, and thus be found throughout the population, a process that has been mathematically supported for three types of polygamous mating systems (*15*).

In addition to avoiding the objections to an adaptive response through natural selection, the evolution of lepidopteran male pheromones through sexual selection is also supported by other lines of evidence. First, male scent–emitting structures exhibit striking morphological diversity across the Lepidoptera, especially compared to female scent structures; they typically appear sporadically within a taxa and their relative complexity bears little relation to lepidopteran phylogeny (*16*). Second, male pheromones usually (although not universally) are active over only a short range (*7*), making them an energy-inefficient mechanism for reproductive isolation. If natural selection were the driving force behind the species-specific nature of mating systems, one would expect selection for a more energy-conservative long-distance mechanism. Furthermore, as in other groups, there is no evidence for reproductive character displacement, as no intraspecific variance in male structures for scent emission has been reported; throughout the range of a species, these structures appear to be either present or absent. Finally, that sexual selection can operate in moth mating systems has been demonstrated in *Ephestia elutella*. Females of this species not only show a mating preference for larger males, but this preference is apparently based on the fact that large males produce significantly more pheromone (*17*).

In summary, evidence from surveys of host-plant associations and geographical distributions provides independent measures of interspecific contact. Both suggest the evolution of male pheromones in Lepidoptera as reproductive isolating mechanisms due to an adaptive response to interpopulational mating mistakes. The survey format encompassing a large number of species suggests that our conclusions are robust for the Lepidoptera and avoids the difficulty of sampling bias possible in studies of individual species pairs. Although these data do not distinguish between the two adaptive response models presented, the sexual selection model is more consistent with other lines of evidence.

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Direct Activation of Mammalian Atrial Muscarinic Potassium Channels by GTP Regulatory Protein G_k

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The mammalian heart rate is regulated by the vagus nerve, which acts via muscarinic acetylcholine receptors to cause hyperpolarization of atrial pacemaker cells. The hyperpolarization is produced by the opening of potassium channels and involves an intermediary guanosine triphosphate–binding regulatory (G) protein. Potassium channels in isolated, inside-out patches of membranes from atrial cells now are shown to be activated by a purified pertussis toxin–sensitive G protein of subunit composition αβγ, with an α subunit of 40,000 daltons. Thus, mammalian atrial muscarinic potassium channels are activated directly by a G protein, not indirectly through a cascade of intermediary events. The G protein regulating these channels is identified as a potent G_k; it is active at 0.2 to 1 p*M*. Thus, proteins other than enzymes can be under control of receptor coupling G proteins.

MUSCARINIC AGONISTS, SUCH AS acetylcholine (ACh) or carbachol, attenuate adenylyl cyclase activity in heart membranes (*1, 2*) and hyperpolarize atrial cells by opening potassium channels (*3, 4*). Both effects depend on a guanine nucleotide–binding coupling (G) protein (*1, 5*) and are blocked by pertussis toxin (PTX) (*6, 5*). However, the two effects do not appear to be causally related, because adenylyl cyclase activity can be inhibited in isolated depolarized membranes (*1, 2*) and cyclic nucleotides do not affect opening of K^+ channels (*7*). Indeed, two types of electrophysiological experiments suggest that ACh-mediated opening of K^+ channels does not require generation of a soluble second messenger: (i) K^+ channels in a cell-attached "gigaseal" patch cannot be activated by ACh applied to the cell surface outside the patch but open readily upon addition of the neurotransmitter through the patch pipette (*8*); and (ii) addition of a nonhydrolyzable guanosine triphosphate (GTP) analog, guanosine 5′-(γ-thio)triphosphate (GTPγS), to the cytoplasmic face (bath side) of an inside-out patch activates the K^+ channel (*9*). Although the fact that PTX uncouples the muscarinic response suggests the involvement of a G protein of the αβγ type, neither

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