

# Disruptive sexual selection in *Colias eurytheme* butterflies

(pheromone variation/alkanes/courtship)

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**ABSTRACT** Sexual selection on male pheromone composition in *Colias eurytheme* (Pieridae) butterflies has the remarkable effect of increasing the variability of this trait. Sexual selection on important traits is generally thought to have a strong stabilizing effect on intraspecific variation of those characters. In this species, however, the male courtship pheromone is highly variable in the relative proportions of its three chemical constituents. Stabilization and/or canalization of this polygenic character in a population is impeded by the disproportionate mating success of males in one portion of the character distribution with alba (white morph) females, and of those in the opposite portion with orange (colored morph) females.

Ultraviolet reflectance and correct courtship pheromone are the cues by which a *Colias eurytheme* female assesses the conspecificity of a courting male butterfly (1, 2). The pheromone, located on the dorsal surface of the hindwing (1), consists of the common cuticular hydrocarbons *n*-heptacosane ( $C_{27}$ ), 13-methylheptacosane (13-Me $C_{27}$ ), and possibly *n*-nonacosane ( $C_{29}$ ) (3). Virgin females often reject one or more conspecific males before accepting one (4), suggesting the possibility of intraspecific discrimination among potential mates by the female. Rejection or acceptance does not depend on previous experience of the female, but rather, by inference (5), on internal threshold(s) probably involving several hierarchically arranged cues (6). Age, size, wing wear, courtship persistence (4), and phosphoglucose isomerase (PGI) genotype (7, 8) have all been implicated in the differential mating success of males, but correlated differences in the quality or quantity of the courtship pheromone have been suggested as a possible proximate factor influencing female mate choice (4, 8).

Within populations of *C. eurytheme*, the male pheromone is extraordinarily variable in both the quantities and relative proportions of the three components ( $C_{27}$ , 13-Me $C_{27}$ , and  $C_{29}$ ) (9, 10). Part of this variation is polygenically controlled, and the pheromone phenotype of an individual remains remarkably constant throughout the life of the adult, although it does change somewhat as wing wear increases with age (9, 10). We hypothesized that during courtship, females assess the pheromone configuration of conspecific males and that certain pheromone profiles are preferred over others.

## MATERIALS AND METHODS

Pheromones of individual males were analyzed by gas chromatography as described (9). Because the three chemicals making up the pheromone are genetically and phenotypically correlated (9), and because of the peculiar statistical properties of ratios (11), principal components were drawn from the data and used to examine pheromone configurations (Table 1). The principal components of  $C_{27}$ , 13-Me $C_{27}$ , and

$C_{29}$  quantities were extracted using a correlation matrix for each population separately. In all but one case (Crystal Lake), axis 1 has relatively high positive loadings for all three hydrocarbons which are similar in magnitude to one another, and it therefore represents a general quantity axis. The remaining two axes have eigenvalues less than 1.0, but this is not important in this case. Bioassays have shown that females tend to respond positively to 13-Me $C_{27}$  and negatively to  $C_{27}$  and  $C_{29}$  (3). Thus, we were seeking an axis contrasting 13-Me $C_{27}$  to the straight-chain hydrocarbons regardless of the contribution of this contrast to overall pheromone variation. There are two major categories of 13-Me $C_{27}$ / $C_{27}$ - $C_{29}$  contrast eigenvectors that fall out as either the second or the third principal component. Type I is found in four populations and represents a trend toward negative covariation between 13-Me $C_{27}$  and  $C_{29}$ . Type II is found in three populations and represents a trend for 13-Me $C_{27}$  to increase by a factor of 2 for a unit decrease in  $C_{27}$  and  $C_{29}$ . A third type of contrast is exhibited by the Crystal Lake population, in which 13-Me $C_{27}$  negatively covaries with  $C_{27}$ .

Factor scores along the quantity axis and the axis contrasting 13-Me $C_{27}$  to one or both of the *n*-alkanes were compared between males collected in random sweep-net samples and those collected *in copula* with both alba (white morph) and orange (colored morph) females (Table 2, Figs. 1 and 2). Specific dates, times, and methods of collection can be found elsewhere (1, 9, 10). Extraction and chromatography of pheromone from males captured and stored since 1966-1968 showed that little volatilization of pheromone components had occurred (6). This is not surprising when one considers the high molecular weights of the pheromone components (3), nor is such stability unprecedented (12, 13). Although some degradation or volatilization of the pheromone components may have occurred in the stored *Colias* specimens, this should not bias any conclusions reached, as long as the process of degradation does not differ among mated males and males from random samples, a reasonable assumption.

## RESULTS AND DISCUSSION

In populations where trends were detected for scores along the contrast axis, alba-mated males have higher factor scores than random males, orange-mated males have lower factor scores than random males, and alba-mated males have higher factor scores than orange-mated males (Table 2). A high factor score reflects a high 13-Me $C_{27}$ / $C_{27}$  and/or  $C_{29}$  ratio. This pattern of mating success seems to represent an example of disruptive sexual selection, in which males from one portion of the frequency distribution mate with females of one morph, while males in the opposite portion mate with females of the other morph, albeit with large overlap of the two portions. These tendencies were found in widely sepa-

Abbreviations:  $C_{27}$ , *n*-heptacosane; 13-Me $C_{27}$ , 13-methylheptacosane;  $C_{29}$ , *n*-nonacosane; PGI, phosphoglucose isomerase.

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Table 1. Eigenvectors, eigenvalues, and hypothetical eigenvectors for principal components (PC) analyses of pheromone components from eight populations of *C. eurytheme*

Population	Pheromone component	Eigenvectors*			PC type	Hypothetical eigenvectors		
		PC1	PC2	PC3		PC1	PC2	PC3
Chandler, Maricopa County, AZ (1968)	C <sub>27</sub>	0.652	-0.016	-0.758	I	1	0	-2
	C <sub>29</sub>	0.543	-0.688	0.482		1	-1	1
	13-MeC <sub>27</sub>	<u>0.530</u>	<u>0.725</u>	<u>0.440</u>		1	1	1
		1.877	0.772	0.351				
Antelope, Yuma County, AZ (1988)	C <sub>27</sub>	0.602	-0.118	-0.790	I	1	0	-2
	C <sub>29</sub>	0.573	-0.626	0.529		1	-1	1
	13-MeC <sub>27</sub>	<u>0.557</u>	<u>0.771</u>	<u>0.309</u>		1	1	1
		1.804	0.654	0.541				
Wellton, Yuma County, AZ (1988)	C <sub>27</sub>	0.489	0.872	-0.009	I	1	2	0
	C <sub>29</sub>	0.616	-0.352	-0.705		1	-1	-1
	13-MeC <sub>27</sub>	<u>0.618</u>	<u>-0.339</u>	<u>0.710</u>		1	-1	1
		2.027	0.621	0.618				
Lawrence, Douglas County, KS (1988)	C <sub>27</sub>	0.553	0.832	0.047	I	1	2	0
	C <sub>29</sub>	0.587	-0.429	-0.686		1	-1	-1
	13-MeC <sub>27</sub>	<u>0.591</u>	<u>-0.352</u>	<u>0.726</u>		1	-1	1
		2.221	0.463	0.316				
El Centro, Imperial County, CA (1988)	C <sub>27</sub>	0.567	0.708	-0.422	II	1	1	-1
	C <sub>29</sub>	0.567	-0.707	-0.424		1	-1	-1
	13-MeC <sub>27</sub>	<u>0.598</u>	<u>-0.001</u>	<u>0.801</u>		1	0	2
		2.244	0.449	0.307				
Tempe, Maricopa County, AZ (1967)	C <sub>27</sub>	0.629	-0.231	-0.742	II	1	-1	-1
	C <sub>29</sub>	0.600	-0.464	0.652		1	-1	1
	13-MeC <sub>27</sub>	<u>0.495</u>	<u>0.855</u>	<u>0.153</u>		1	2	0
		2.107	0.654	0.239				
Orchard Lake, Scott County, MN (1966)	C <sub>27</sub>	0.602	-0.309	-7.36	II	1	-1	-1
	C <sub>29</sub>	0.591	-0.447	0.671		1	-1	1
	13-MeC <sub>27</sub>	<u>0.537</u>	<u>0.839</u>	<u>0.086</u>		1	2	0
		2.591	0.360	0.049				
Crystal Lake, Dakota County, MN (1966)	C <sub>27</sub>	0.676	-0.236	-0.698	III	1	0	-1
	C <sub>29</sub>	0.266	0.962	-0.067		0	1	0
	13-MeC <sub>27</sub>	<u>0.687</u>	<u>-0.141</u>	<u>0.713</u>		1	0	1
		1.792	0.943	0.265				

\*Eigenvalue for each set of eigenvectors is given below the line.

rated geographic areas, with significant ( $P < 0.05$ ) trends found in five populations, and a consistent and nearly significant ( $0.05 < P < 0.15$ ) trend found in another.

The adaptive significance of this pattern of sexual selection remains obscure. Although males at the extremes of the pheromone character distribution are acceptable to females of one or the other morph, it is not clear that the females of either morph gain a fitness advantage by selecting such males or by

rejecting males from the "wrong end" of the spectrum. This sexual selection may be nothing more than a manifestation of physiological differences between the alba and colored morphs, perhaps a pleiotropic effect of the alba allele.

The female-limited alba dimorphism is widespread throughout the genus *Colias* (14). Alba and orange females differ physiologically in several important ways (15, 16), and it is possible that the two phenotypes have different thresh-

Table 2. Overview of strength and direction of significant differences (Mann-Whitney *U* tests) in factor scores from principal components (PC) analyses of pheromone component quantities for mated (M), alba-mated (A), orange-mated (O), and random (R) males

Population	PC type	PC1 (quantity)				PC2 or PC3 (13-MeC <sub>27</sub> vs. C <sub>27</sub> -C <sub>29</sub> )			
		M-R	A-R	O-R	A-O	M-R	A-R	O-R	A-O
Antelope, AZ	I	→	>	=	=	→	=	=	→
Wellton, AZ	I	>	→	→	=	>	>	=	→
Lawrence, KS	I	<	.	.	.	=	.	.	.
Chandler, AZ	I	>>>	=	>>>	<<<	=	>	←	>>>
El Centro, CA	II	=	=	=	=	=	=	=	=
Tempe, AZ	II	<	<<<	=	<<<	←	=	<<	>>
Orchard Lake, MN	II	<<	.	.	.	<<	.	<<	.
Crystal Lake, MN	III	←	=	←	=	<	=	<	=

>>>, Significant difference at 0.001 level; >>, significant difference at 0.01 level; >, significant difference at 0.05 level; →, nonsignificant trend at 0.15 level; =, nonsignificant difference; ., insufficient sample sizes.

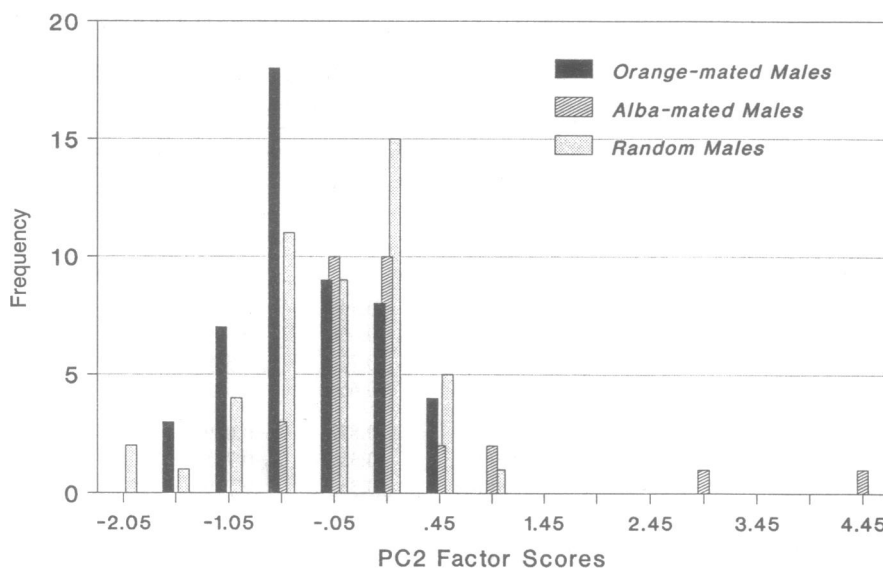


FIG. 1. Frequency distributions for factor scores along principal components axis 2 (PC2; 13-MeC<sub>27</sub>/C<sub>27</sub>-C<sub>29</sub> contrast axis) for pheromone on wings of male *C. eurytheme* collected in mated pairs and in random sweep-net samples, Chandler, AZ, August 1968.

olds of pheromone detection that vary depending on environmental conditions. Such thresholds in moths are known to vary with temperature at the level of the central nervous system (17, 18). Thresholds of perception, for example for 13-MeC<sub>27</sub>, in alba females may differ from those of orange females at a given temperature. Thus, when alba body temperatures are outside the range optimal for 13-MeC<sub>27</sub> perception, greater than average 13-MeC<sub>27</sub> in the pheromone may be required to render a male acceptable, whereas average males might be acceptable to orange females because more of the 13-MeC<sub>27</sub> is detected. Such differential effects of temperature on thresholds of perception could explain why the pheromone distribution of alba-mated males is significantly different from the random distribution in some populations whereas orange-mated males are significantly different in others (Table 2), the difference in observed mating patterns possibly being the result of differences in climatic and microclimatic variables at the time of mated-pair collection. Male *Colias* mating success does vary with microclimatic conditions with regard to PGI genotype (7, 8), but it is

not known whether PGI and pheromone phenotype are correlated; indirect evidence suggests they are not (10).

It appears that much of the high pheromone variation observed in *C. eurytheme* populations (9) is maintained by the opposing selective pressures exerted by the two female morphs. This is quite an extraordinary consequence of sexual selection on the variation of an important fitness character, in this case pheromone composition, which one would expect to be stabilized and/or canalized rather than increased by mate choice (19). The next step will be to determine in algebraic terms whether the observed selective pressures are sufficient by themselves to maintain a stable polymorphism.

We must also now consider the intriguing possibility that a male's mating success is determined by its phenotype within at least three different spheres at adaptation: size (4), PGI genotype/courtship persistence (4, 7, 8), and pheromone composition. A coherent understanding of this species' complex mating system will require integration of knowledge regarding all of these spheres.

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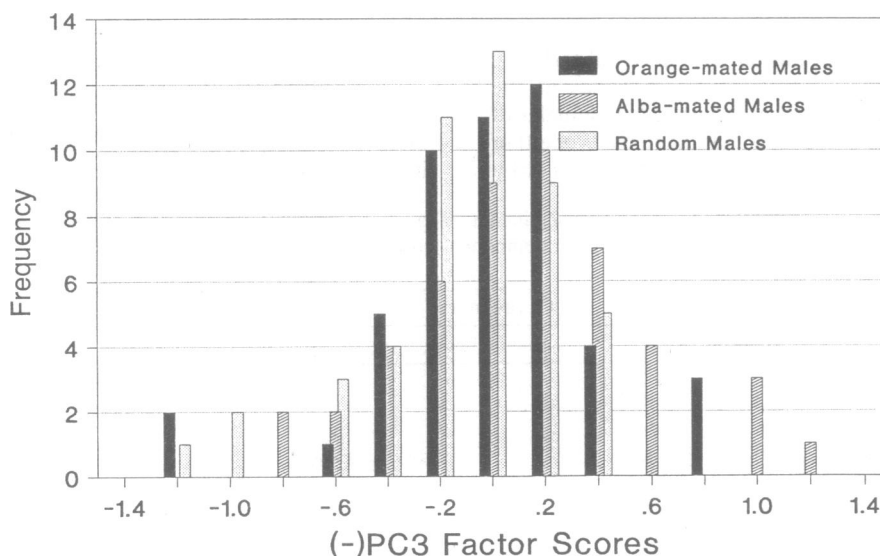


FIG. 2. Frequency distributions for factor scores along principal components axis 3 (PC3; 13-MeC<sub>27</sub>/C<sub>27</sub>-C<sub>29</sub> contrast axis) for pheromone on wings of male *C. eurytheme* collected in mated pairs and in random sweep-net samples, Wellton, AZ, August 1988.

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