

Metabolic resource allocation vs. mating attractiveness: Adaptive pressures on the “alba” polymorphism of *Colias* butterflies*

(microevolutionary genetics/behavioral vs. physiological effects/mass-energy-time budget)

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ABSTRACT The sex-limited “alba” genetic polymorphism in wing color of *Colias* butterflies has been studied with respect to potential selective pressures on this locus. Alba female pupae, carrying at least one dominant *A* allele, redirect resources, used by *aa* pupae for pigmentation, to other metabolic ends. Associated with this reallocation, alba, *A*–, female adults eclose earlier, retain more larva-derived resources in their fat bodies for somatic maintenance and for reproduction, and, in some conditions, mature their eggs faster than do *aa* females. Alba females are also less attractive to males than are *aa* females and mate less frequently. Evolutionary implications of these results are discussed.

Most species of sulfur butterflies, *Colias* (Lepidoptera, Pieridae), show the “alba” genetic polymorphism, sex-limited in expression, for adult pteridine wing pigmentation. Females carrying a dominant allele *A* at a single autosomal locus are white instead of the species-specific red, orange, or yellow displayed by their homozygous recessive *aa* sisters and by all males. This genetic mechanism, first demonstrated by Gerould (1, 2), is the same throughout the genus (3, 4). North American *Colias* have higher *A* frequencies in colder-habitat populations although, at any one place, sympatric species may have quite different frequencies (5, 6).

A sufficient evolutionary explanation of this polymorphism would include: (a) analyzing the mechanistic basis of the phenotypic differences among genotypes; (b) identifying the selective pressures acting on the phenotypic differences; (c) determining the mode of selection (heterosis? frequency dependence? etc.) and the importance of factors such as genetic drift; and (d) estimating selection coefficients in experiments or natural conditions. *a* and *b* must precede *c* and *d*, both logically and to restrict the latter tasks to a feasible scale.

Descimon (7) and Watt (8) began study of the biochemical mechanism of alba. They found that alba females sharply decrease the input of precursor to wing pteridine synthesis. This shifts the kinetic balance of the pathway so that only colorless pteridines accumulate in the wings. This diversion of nitrogen-rich pigment material to other uses in the closed metabolic system of the developing pupa may be a major advantage of alba in the wild (8), especially since herbivorous insects in general (e.g., refs. 9 and 10), Lepidoptera (e.g., refs. 10, 11, and 12), and *Colias*' close relative *Pieris* most particularly (10) are nitrogen-limited in growth. An early hypothesis (13) that *A*– females fly in cooler parts of the day than do *aa* females, or otherwise differ in their adult thermal biology, has been refuted by field behavioral (14) and thermal-ecological (8) studies. It has been suggested that the morphs might be differently attractive to males because of their color difference (8). Evidence to test these ideas further and to integrate laboratory and field perspectives has been lacking until now.

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Here we report field study of two *Colias* species—*C. alexandra* Edwards and *C. scudderi* Reakirt—in different species complexes of the genus. These are broadly sympatric in the southern Rocky Mountains but differ in many aspects of habitat use (15, 16) and display very different *A*– and *aa* morph frequencies. We also report laboratory studies of a third species, *C. eurytheme* Boisduval, which complement the field results. These comparisons allow search for general differences between the morphs in different microhabitat contexts and against phylogenetically distinct genetic backgrounds.

MATERIALS AND TECHNIQUES

Study Animals. *C. alexandra* was studied in a fescue grassland meadow (17) at 2865-m (9400 feet) elevation in a tributary valley of the East River (Gunnison County, CO) near the junction of West Brush and Brush Creeks. *C. scudderi* was studied in a willow marsh at 2725-m (8940 feet) elevation adjacent to the East River, east of Crested Butte Mountain, about 2.5 km from the *C. alexandra* site. General ecology and population structure are as reported for these species in this drainage (16).

Our laboratory colony of *C. eurytheme* at Stanford was used as a source of broods segregating *Aa* and *aa* genotypes. Larvae were reared in a 16-hr-light/8-hr-dark photoperiod, at 25°C by day and 21°C by night; vetch (*Vicia*) was used as larval food.

We used proven methods (16, 18) to assess age of field-caught *Colias* by rating physical wear on the wings. The few extremely worn (class 5) individuals taken were lumped with worn (class 4) individuals. Wear classes were: 1, freshly eclosed, cuticle incompletely tanned (none found in this work); 2, scale pattern and wing intact, no wear; 3, moderate scale erosion and cutting or tearing of wing; 4, severe to extreme scale erosion and laceration of wing.

Frequencies of alba, *A*–, and yellow, *aa*, morphs were assessed in the field by random hand-net sampling. Individuals were taken from these samples for wear rating and laboratory dissection. At times those individuals released were felt-pen marked for recapture estimation of female numbers. We used for this the Lincoln index procedure (19) which is less accurate than our usual (16, 18) computerized implementation of Jolly's stochastic methods (20) but is less time-consuming.

Female Mating Frequency, Nutrient Storage, and Egg Maturity. During mating, a male butterfly deposits a proteinaceous spermatophore in the female's bursa copulatrix (21).

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This is a vehicle for sperm transfer and may serve as a source of female nutrition (22). Absorption of this material by the female may, in some taxa, entirely destroy spermatophore structure (23, 24); in others, each spermatophore remains recognizable through the female's life-span (24, 25). The latter case holds for *Colias*; thus, we could dissect a female and, by counting spermatophores in the bursa, obtain an accurate count of female matings in the manner of Burns (25). Multiple mating is a routine part of the reproductive strategy of *Colias* (unpublished data).

The fat body of an adult female butterfly at emergence contains nutritional reserves carried through pupation from larval feeding. These are a major part of the resources available for somatic maintenance and gamete production. They are important in *Colias* as a source of gamete nitrogen, as shown by isotope studies of the relative carryover of larval amino acid and larval carbohydrate pools to adult egg production (unpublished data). The mass of the fat body decreases as reserves are used. At dissection for spermatophore counting, each female's fat body was rated on this scale: 4, abdomen packed with fat body, viscera and tracheal network buried; 3, viscera not buried, tracheae obscured, fat body mass 0.75 that of class 4; 2, most remaining fat body adherent to tracheal net, fat body mass 0.5 that of class 3; 1, little or no remaining fat body.

To avoid observer bias when testing association of fat body state, mating frequency, and wing wear, one of us first rated each female for wing wear, opened the abdomen and removed the bursa for spermatophore counting, and then passed the abdomen to another of us for fat body rating. Ratings and counts were recorded at each stage but not exchanged until each animal was fully scored.

In one experiment, maturity of egg development in freshly emerged females was rated on this scale: 5, ribs visible on chorion, mature size and taper of egg, nurse cells atrophied or absent; 4, chorion immature, egg full size but not tapered, nurse cells 10–20% of egg mass; 3, egg size 50% of maturity, nurse cells >20% of egg mass; 2, egg size 25% of maturity, nurse cells about 50% of egg mass; 1, egg size <25% of maturity, nurse cells not yet distinct from egg, yolk deposition begun; 0, yolk deposition not yet begun.

Tests of Female Attractiveness to Males in the Field. A fuller evaluation of methods for assessing male–female interactions in *Colias* in the field will appear elsewhere. Briefly, wild-caught females were tethered so as to rest, visually ex-

posed, on the ground or at the top of vegetation but below the flight plane of freely flying males in the study environment. One fresh A– and one fresh *aa* morph were tethered about 10 cm apart in each trial, providing a paired stimulus to test male preferences. Males flying through the test area might ignore the test, show “investigative dip” behavior, or visit one of the females. A “dip” consists of marked flight deviation to within 0.2–0.4 m of the investigated object(s) followed by departure with no further interaction. A “visit” consists of an approach to within 1–3 cm of a test female at least 2 sec in duration (frequently longer) and usually with active male courtship of the visited female. The distinction between these behaviors was sharp; on cross-checks at intervals, we always agreed with one another on scoring. Occasional visits resulted in mating between visiting males and test females.

Yellow and white fabric flags, 3 × 3 cm on 10-cm wire supports, were also used in paired tests of males' visual reactions. Males would dip toward or visit these as they do females, but visitation was not as prolonged with flags as with females.

Data Reduction. Most statistical work was done by standard methods (26–28). Multiway analysis of variance with unequal sample sizes was done by a modification of Veldman's (29) procedure. All calculations, except for Wilcoxon matched pair rank tests, were done with BASIC and FORTRAN IV programs on a NOVA 2/10 minicomputer.

RESULTS

Densities, Morph Frequencies, and Morph Wear Ratings in the Field. Estimated numbers of females in the study populations were: *C. alexandra*, 540 on 12–13 July 1978, 418 on 17–18 July 1978; *C. scudderii*, 90 on 25–26 July 1978, 252 on 26–27 July 1978, 220 on 27–28 July 1978. These data are consistent with densities of a few per hectare found earlier (16).

The population morph frequencies over the whole season were: *C. alexandra*, $f_{A-} = 0.126$, $f_{aa} = 0.874$; *C. scudderii*, $f_{A-} = 0.812$, $f_{aa} = 0.188$.

We wished to examine in the wild the suggestion (8) that pupal resource reallocation might lead to shorter pupal development time, and hence earlier eclosion, in A– than in *aa* females. One might detect such an effect *via* morph frequency changes through the flight season, but observed population densities did not allow large enough samples to detect any but the largest possible changes. The fact that average wing wear is a reliable indicator of sample age in these and other *Colias*

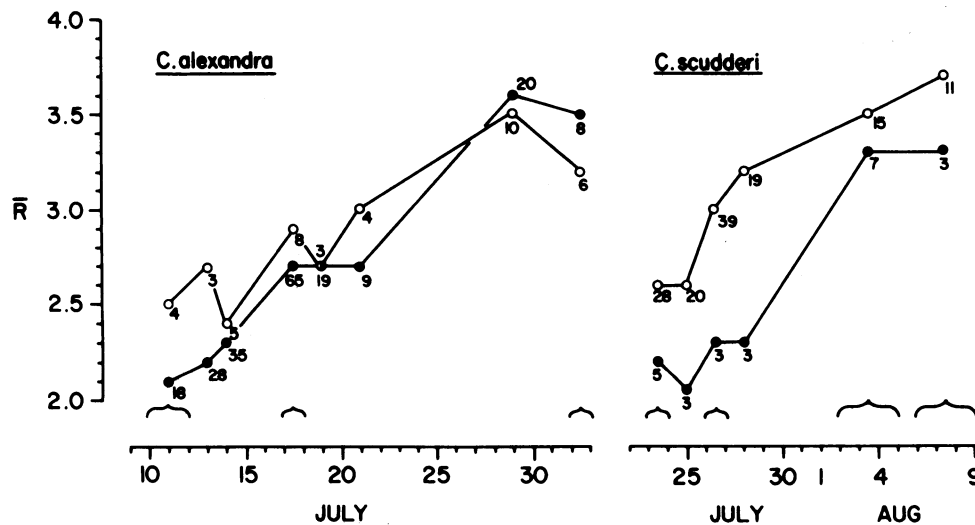


FIG. 1. Average wear ratings, \bar{R} , a measure of average age (16, 18) plotted against time in 1978 for A– (O) and *aa* (●) females of *C. alexandra* and *C. scudderii*. Numbers near points are numbers of animals averaged. If, on a given sampling day, either morph was represented in our sample by fewer than three individuals, that sample was pooled, morph by morph, with the sample nearest in time; no more than three samples were pooled together and no more than four calendar days ever were spanned, as shown by brackets in the figure. Samples not meeting these restrictions were discarded. Wilcoxon matched pair rank tests for higher average wear in A– females: *C. alexandra*, 10–21 July, T_s (Wilcoxon score) = 0, $df = 5$, $P = 0.031$, 10 July–2 August, $T_s = -6$, $df = 7$, $0.05 < P < 0.10$; *C. scudderii*, 23 July–9 August, $T_s = 0$, $df = 6$, $P = 0.016$.

Table 1. Effects of age and female morph on fat body reserves and mating numbers in female *Colias*

	Wing wear classes			Analysis of variance			
	2	3	4	Effect	F	df	P
Spermatophore counts							
<i>C. alexandra</i> :							
A-				Morph	18.0	1,	<0.001
Mean	1.05	1.44	1.90			137	
n	15	23	13	Wing wear	30.2	2,	<0.001
aa				Inter-action	0.4	2,	0.66
Mean	1.34	1.91	2.50			137	
n	31	41	20				
<i>C. scudderii</i> :							
A-				Morph	5.4	1,	0.02
Mean	1.05	1.55	1.81			93	
n	17	27	28	Wing wear	18.2	2,	<0.001
aa				Inter-action	0.1	2,	0.90
Mean	1.23	1.88	2.15			93	
n	12	9	6				
Fat body ratings							
<i>C. alexandra</i> :							
A-				Morph	16.4	1,	<0.001
Mean	3.92	2.91	1.64			127	
n	12	22	11	Wing wear	74.2	2,	<0.001
aa				Inter-action	1.4	2,	0.24
Mean	3.34	2.03	1.35			127	
n	29	39	20				
<i>C. scudderii</i> :							
A-				Morph	9.7	1,	0.003
Mean	3.82	2.78	1.76			94	
n	17	27	29	Wing wear	50.0	2,	<0.001
aa				Inter-action	1.3	2,	0.28
Mean	3.42	1.89	1.50			94	
n	12	9	6				

Age, indexed by wing wear, increases from class 2 to class 4; fat body mass decreases from class 4 to class 1. Each spermatophore represents one mating. Two-way analysis of variance was done on square-root-transformed spermatophore counts as appropriate to a discrete-count variable (26). Fat body ratings were analyzed untransformed as lumped classes of a continuous distribution; the arcsin-square-root transform for percentages of a normalized total, another possible view of the data, gave the same conclusions.

species (16, 18) gave us a sound, albeit indirect, way to address this question: earlier eclosion by one morph would lead to higher average wear ratings for that morph, other things being equal. This difference might lessen as the season progressed and both morphs accumulated more wear. In both species, A- females showed greater wear, consistent with greater age, early in the season (Fig. 1). Later, the difference vanished in *C. alexandra* but persisted through the season in *C. scudderii*, suggesting a greater eclosion difference between morphs in the latter species.

Fat Body State and Mating Frequency of Morphs in the Field. Table 1 presents data and analysis of variance relating to fat body state and multiple mating by both morphs. In both species, fat body decreased and mating number increased consistently with age as indexed by wing wear. In both species and in each wear class, A- morphs had greater fat body reserves but fewer matings than did aa morphs. Differences in mating number were less in *C. scudderii* (average, 19% advantage for aa) than in *C. alexandra* (average, 31% advantage for aa).

Female Morph Attractiveness to Males in the Field. Table 2 presents the results of our female tethering experiments. In every trial for both species, aa females were visited more than

were A- females. This male preference was far more pronounced in *C. alexandra*, which had a low A- frequency, than in *C. scudderii*, which had a high A- frequency.

Colias males approach and investigate objects, such as our flags, with only general resemblance to females. The attraction difference between morphs must be visually based, because the proportions of white and yellow flags visited were extremely close to the proportions of white and yellow morphs visited in each species.

Between morphs for either species, there was no difference in the fraction of male visits lasting more than 5 sec ("long" visits). Once males are in the visit range, 1-3 cm, behavioral cues unrelated to the morph color difference govern courtship. But, males must be attracted within this range by visual stimulus before courtship can proceed, and the color difference thus is isolated more sharply as the source of the difference in male visitation.

Development Rates and Gamete Maturation of Morphs in the Laboratory. Because neither *C. alexandra* nor *C. scudderii* was available in laboratory culture, we reared *C. eurytheme* to test further the idea that A- females may develop faster, or mature their eggs sooner, than aa females (above data and ref. 3). Comparison of Aa and aa females was done with backcross broods segregating these genotypes 1:1, so as to randomize other heritable variation affecting pupal development rate and egg maturation. Table 3 presents the experiment. In a warm thermal regimen, Aa pupae had a slight (2%) advantage of development rate over aa pupae but no consistent advantage in stage of the most mature egg at emergence. In a cold regimen, both genotypes had extended development times, Aa showing 4% development rate advantage over aa and Aa females emerging with significantly more mature eggs than aa females. Thus, Aa have a greater physiological advantage over aa under cold than under warm conditions.

Table 2. Visits by male *Colias* to paired white (A-) and yellow (aa) female morphs and to paired white (W) and yellow (Y) flags

Day	Time	<i>C. alexandra</i>				<i>C. scudderii</i>			
		Morphs		Flags		Morphs		Flags	
		A-	aa	W	Y	A-	aa	W	Y
1	AM	5	12	1	16	11	15	10	13
	PM	0	3	2	10	12	17	6	11
2	AM	5	19	9	26	8	9	11	16
	PM	0	4	2	16	27	33	9	24
3	AM	2	12	4	23	3	4	7	11
	PM	0	2	1	10	34	47	12	13
4	AM	3	12	5	18	8	13	8	12
	PM	0	8	1	13	18	28	13	15
5	AM	7	27	11	54	—	—	13	14
	PM	0	5	0	10	—	—	13	19
6	AM	3	9	3	16	—	—	—	—
	PM	0	5	3	12	—	—	—	—
Total visits		25	118	12	216	121	166	102	148
Visit ratios		0.21:1		0.19:1		0.73:1		0.69:1	
Long visits		6	26	—	—	49	62	—	—

AM = 1000-1200 hr; PM = 1201-1400 hr. All comparisons were evaluated with *G* tests using Yates' correction and *df* = 1. In both species, males preferred yellow females or flags to white females or flags. For *C. alexandra*: morphs, *G* = 64.2, *P* < 0.001; flags, *G* = 135.1, *P* < 0.001. For *C. scudderii*: morphs, *G* = 6.8, 0.005 < *P* < 0.01; flags, *G* = 8.1, 0.001 < *P* < 0.005. In each species, the ratios of visits to yellow and white morphs or flags were the same. For *C. alexandra*, *G* = 0.09, 0.7 < *P* < 0.8; for *C. scudderii*, *G* = 0.05, 0.8 < *P* < 0.9. Neither species showed difference between morphs in the proportion of long visits (5 sec) to total visits. For *C. alexandra*, *G* = 0.03, *P* > 0.9; for *C. scudderii*, *G* = 0.17, 0.5 < *P* < 0.7.

Table 3. Pupal development and egg maturation of *Aa* and *aa* females of *Colias eurytheme* in two thermal regimes

Brood	t_{Aa}	n_{Aa}	t_{aa}	n_{aa}	E_{Aa}	E_{aa}
Warm regimen						
36	141	2	140	2	2.0	2.5
37	152	7	156	11	3.4	3.2
39	148	3	149	5	3.0	3.0
59	147	7	154	3	3.6	3.3
94	153	3	156	7	3.3	3.7
96	153	3	157	3	2.6	2.0
103	147	3	149	3	2.6	2.3
119	158	5	162	2	2.4	2.0
Cold regimen						
37	1108	6	1108	3	3.5	3.3
39	1239	4	1262	5	3.0	1.6
59	1202	6	1279	4	3.2	2.3
83	1247	4	1269	2	2.3	2.0
96	738	2	805	7	2.5	2.0
119	972	9	1024	7	3.0	2.3

Warm regimen was a 16-hr day at 25°C and an 8-hr night at 21°C; cold regimen was an 11-hr day at 15°C and a 13-hr night at 5°C. Larvae were reared to pupation under standard conditions; pupal development time, t , was scored in hours from pupal to adult molts; egg maturity at adult molt, E , was scored from 5 (most mature) to 0 (least mature). Wilcoxon matched pair rank tests were used in the following statistical evaluations (T_s , Wilcoxon score). t_{Aa} vs. t_{aa} : warm regimen, $T_s = 1\frac{1}{2}$, $df = 8$, $0.008 < P < 0.012$; cold regimen, $T_s = 0$, $df = 5$, $P = 0.031$. E_{Aa} vs. E_{aa} : warm regimen, $T_s = 10\frac{1}{2}$, $df = 7$, $0.29 < P < 0.34$; cold regimen, $T_s = 0$, $df = 6$, $P = 0.016$.

DISCUSSION

We now have positive evidence for expression of the resource allocation advantage of alba in eclosion time, egg maturation, and fat body state and for its mating attractiveness disadvantage. The effects seen appear to be general across species complexes, albeit modified in detail by particular circumstances.

The diversion of material from pteridine synthesis in the pupa could result from changed kinetics or concentration of GTP cyclohydrolase, the enzyme that synthesizes the pteridine ring. It is equally likely to reflect a change at some locus earlier in purine nucleotide metabolism, lowering the pool concentration of GTP in its role as pteridine precursor. Descimon (30) has argued for the latter upon finding (in *C. croceus* Fourcroy) that *Aa* females differ from *aa* in isotope incorporation from guanosine into excreta as well as into wing pigments. But the amount of material diverted from the wing pigments is such [0.25% of female wet weight, several percent of the animal's nitrogen budget (8)] that a block of this magnitude at either location could equally well cause shifts in the kinetic balance of purine metabolism throughout the pupa. Only specific enzymologic and radioisotopic pathway analysis can identify positively the locus of alba action.

Faster development will lead to reproductive onset by alba earlier in the population's flight period. This will mean time for more offspring larvae to attain the minimal size needed to survive larval diapause, before cold or drying up of larval food-plant foliage put an end to effective larval feeding (31). In warmer-habitat multivoltine *Colias*, reproductive onset early in flight periods will result in a higher rate of increase, r , for *A*—as the broods progress toward overlapping generations each year.

Faster egg maturation will lead to reproductive onset earlier in individuals' lifetimes for *A*— than for *aa* genotypes. This will give *A*— genotypes a greater opportunity for oviposition. This may be particularly important in that *Colias* displays type II

adult survival: loss rates are roughly constant and quite high, so that few individuals ever attain their physiological maximal life-span. Indeed, female mortalities in excess of 0.5 per day have been measured in some East River drainage *Colias* populations (16, 18). Even a few hours' advantage in reproductive opportunity, in a population with an average life-span of only a few days, may constitute powerful selection.

The difference in fat body reserves between morphs could translate into various selective pressures. Total eggs produced, stored nutrient quality and thus viability of those eggs, or even general viability of adult females themselves, all could be affected. *aa* females might be able to compensate by increased feeding [the adult nectar diet contains both sugars and amino acids (32)], but an increase in feeding time would decrease time available for oviposition, especially in montane or northern habitats in which *Colias* can be active only a few hours each day. Different aspects of *Colias*' mass-energy-time budget could thus be affected by alba in different ways.

The physiological effects of alba are modifiable by environmental conditions: cold increases alba's impact on the pupal resource budget. This impact might be further increased by stress in the larval environment. The development rate, and eventually survival, of *Colias* larvae is reduced dramatically by temperatures below the larval optimum (33); cold might also reduce effectiveness of resource assimilation into the larval fat body, giving the pupa fewer resources for development and increasing further the importance of resources reallocated in alba.

The wide *A* frequency difference between *C. alexandra* and *C. scudderii* may be related to their thermally distinct juvenile microhabitats. *C. alexandra*'s legume food plant occurs, and larvae and pupae thus develop, in dry, exposed grasslands which even in spring (June) are quite warm on sunny days. *C. scudderii* feeds primarily on willows (*Salix*) in marches and stream courses, which serve as channels for cold air flow. Further, *Salix*' dense vegetation holds cold air longer in the day than does the open grassland vegetation. *C. scudderii* thus should mature under more severe cold stress than does *C. alexandra*, and the pupal resource reallocation by alba should be more important to *C. scudderii*.

Silberglied and Taylor (34) concluded that *Colias* males approach females after visual recognition at a distance, and pheromone signals first enter into the courtship sequence when the male is within a few centimeters of the female—what we have scored as a visit. Our results agree fully with this model. Yellow ground color in females [coupled with ultraviolet absorption (34)] seems to represent an optimal visual stimulus for males, even when *aa* females are rare as in *C. scudderii*. Male *C. scudderii* investigate a broader range of stimuli than do male *C. alexandra*. This may have evolved from various causes, distinguishable by further field work.

(a) *C. alexandra* males in their grassland habitats encounter more white non-*Colias* butterflies, notably other pierids, than do *C. scudderii* males in their willow marshes; *C. alexandra* males may be selected to avoid waste of time and energy by ignoring white butterflies more sharply than do *C. scudderii* males.

(b) *C. alexandra* and *C. scudderii* meet each other as adults while feeding, because *C. scudderii* must usually leave its marsh habitats to find nectar sources; the fact that males of the two species react differently to the alba phenotype may function as a partial isolating mechanism, given the interspecific frequency difference.

(c) The physiological advantage of alba may control the balance of allele frequencies in *C. scudderii* so that the male *C. scudderii* search image for females has perforce evolved to greater breadth.

Mating success of the female morphs is not simply proportional to their attractiveness difference. This may be because, except at low population densities, each female will be courted more often than she is receptive. This does not impeach the selective potential of the difference in mating success. Spermatophores represent at least some nutrient transfer in *Colias* as in other butterflies (22; unpublished data), so that even a small mating advantage may represent differential resource input to female longevity and reproduction. The short life-span of adult *Colias* (see above and refs. 16 and 18) means that differences of a few hours before first mating, or in delay before a needed second mating, etc., may have major average effects on the reproductive output of the morphs. *aa* females may also gain in fitness, due to greater offspring variability, by reproducing with a greater variety of mates, but this should be a "second-order" effect.

Is the dominance of *alba* as complete in other effects as it is in pigmentation changes? Remington (4) summarized evidence that *AA* might be less viable than *Aa*, or that the *A* allele might frequently be linked to a recessive subvital allele at some unknown locus. Lorkovicz and Herman (3) did not find this in *C. croceus*. They later reported (34) a population cage experiment that they interpreted as indicating general inviability, at high temperatures, of *A*—compared to *aa*. This interpretation rests on their unsupported assumption that the wild sample used to found the cage population was in Hardy-Weinberg equilibrium. Because *AA* and *Aa* cannot be distinguished visually by humans, their results could equally well have been due to a deficiency of *AA* in the founding sample or a subsequent low viability of this genotype in the cage. A clear and general resolution of the question is needed.

This study serves to emphasize that selection operates on many levels of living organization—from biochemistry to behavior—and that the whole mass-energy-time budget of an organism may be affected in various ways by selection at one locus. Further study of *alba* seems likely to yield further illustration of these ideas and perhaps to illuminate the adaptive organization of the mass-energy-time budget itself.

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