ADAPTATION AT SPECIFIC LOCI. IV. DIFFERENTIAL MATING SUCCESS AMONG GLYCOLYTIC ALLOZYME GENOTYPES OF COLIAS BUTTERFLIES

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Manuscript received May 30, 1984
Revised copy accepted September 20, 1984

ABSTRACT

Male mating success as a function of genotype is an important fitness component. It can be studied in wild populations, in species for which a given group of progeny has exactly one father, by determining genotypes of wild-caught mothers and of sufficient numbers of their progeny. Here, we study male mating success as a function of allozyme genotype at two glycolytic loci in Colias butterflies, in which sperm precedence is complete, so that the most recent male to mate fathers all of a female's subsequent progeny.—For the phosphoglucose isomerase, PGI, polymorphism, we predict mating advantage and disadvantage of male genotypes based on evaluation of their biochemical functional differences in the context of thermal-physiological-ecological constraints on the insects' flight activity. As predicted, we find major, significant advantage in mating success for kinetically favored genotypes, compared to the genotype distribution of males active with the sampled females in the wild. These effects are repeatable among samples and on different semispecies' genetic backgrounds.—Initial study of the phosphoglucomutase, PGM, polymorphism in the same samples reveals heterozygote advantage in male-mating success, compared to males active with the females sampled. This contrasts with a lack of correspondence between PGI and PGM genotypes in other fitness index or component differences.—Epistatic interactions in mating success between the two loci are absent.—There is no evidence for segregation distortion associated with the alleles of either primary locus studied, nor is there significant assortative mating.—These results extend our understanding of the specific variation studied and suggest that even loci closely related in function may have distinctive experience of evolutionary forces. Implications of the specificity of the effects seen are briefly discussed.

DARWINIAN fitness differences are subdivisible into components—genotypic viability and fecundity, gametic selection, etc. Variation of fitness components among genotypes in the wild is important to many aspects of evolutionary study and has received much attention (*e.g.*, DUMOUCHEL and ANDERSON 1968; ANDERSON 1969; PROUT 1965, 1969, 1971a,b; CLEGG, KAHLER and ALLARD 1978). Most such work has studied fitness component differences among genetic variants whose functional effects were unknown. Here, we continue development of a different approach: study of natural variation in organisms for which fitness-related properties can be predicted from differ-

ences in mechanistic function and physiological-ecological performance of the variants, followed by test of the predictions in the field (*e.g.*, WATT 1977; 1983; WATT, CASSIN and SWAN 1983; GRAHAM, WATT and GALL 1980). In this way, mechanistic adaptive properties can be associated with resulting differences in fitness indices (clearly selectable character states), fitness components and eventually net fitnesses. Others are also pursuing aspects of this approach (reviews, *e.g.*, POWERS, DIMICHELE and PLACE 1983; WATT 1984).

When sampled parents and their offspring can both be genotyped, fitness component analysis may be very powerful. For example, females of the livebearing fish Zoarces carry their embryonic young for long periods. From samples of gravid females and one offspring from each female's brood, fitness component differences at polymorphic gene loci have been studied (CHRISTIANSEN and FRYDENBERG 1973; CHRISTIANSEN, FRYDENBERG and SIMONSEN 1977). The sperm of different fathers may mix in a single brood of Zoarces, and thus this work could only estimate differences among male gamete frequencies.

If a creature's breeding system is such that any given brood has only one father, one can estimate genotype frequencies among mating males by sampling females and three or more of each female's offspring (CHRISTIANSEN 1980; OSTERGAARD and CHRISTIANSEN 1981). Such a system is being studied in the shrimp Gammarus (SIEGISMUND 1983; H. R. SIEGISMUND and F. B. CHRISTIANSEN, unpublished data).

Such analysis, alone, cannot make certain that observed fitness differences result from segregation at the locus under study, as distinct from closely linked loci "hitchhiking" with it (THOMSON 1977). Linkage disequilibrium between a neutral and a selected locus is transitory (*e.g.*, CLEGG, KIDWELL and HORCH 1980; ASMUSSEN and CLEGG 1981, 1982), but it could easily occur in a given case. Even careful fitness estimation in laboratory populations (*e.g.*, MARINKOVIC and AYALA 1975a,b), in the absence of other information, is subject to this limitation on its interpretation.

Thus, study of mechanistic differences among genotypes may often be necessary for interpreting differences in fitness components or net fitness. If no major functional differences can be found among genotypes that appear to show fitness differences, then hitchhiking is indicated. But, if differences in fitness itself, or in fitness indices or components, are *predictable* from mechanistic differences among the genotypes in question, then hitchhiking is no longer a credible explanation. For example, at the glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase loci of Drosophila, the agreement among genotypic differences in *in vitro* kinetics, *in vivo* metabolism and net fitness in laboratory populations rules out a major role for hitchhiking in the maintenance of these polymorphisms (BIJLSMA 1978, 1980; BIJLSMA and VAN DELDEN 1977; BIJLSMA and VAN DER MEULEN-BRIJNS 1979; CAVENER and CLEGG 1981; CAVENER 1983; EANES 1984).

A multiallelic polymorphism at the phosphoglucose isomerase (PGI, EC 5.3.1.9) locus of the insect genus Colias has been under study for some time (WATT 1977, 1983; WATT, CASSIN and SWAN 1983). *In vitro* biochemical dif-

ferences among genotypes of alleles 2–5, which are frequent to common in the wild, have been extensively studied. These differences, often related to temperature, were then interpreted in context of the insects' thermal ecology and the thermal dependence of their flight, since flight is a direct fitness index (WATT 1968; KINGSOLVER 1983a,b; KINGSOLVER and WATT 1983). This led to predictions of fitness index/component differences among the PGI genotypes; many of these have been tested and verified in the wild (see PREDICTIONS CONCERNING MATING SUCCESS OF SPECIFIC GENOTYPES, later in this paper).

Using PGI alleles as markers, BOGGS and WATT (1981) found that in multiple matings of Colias, sperm precedence is complete. A female's most recent mate fathers all of her eggs thereafter—previous mates' sperm never reappear. Thus, eggs laid by a female in a given period have *one* father. We have used this fact, and the ease of rearing many progeny from wild-sampled female Colias, to study male mating success differences among Colias allozyme genotypes. Rather than confine ourselves to estimating overall genotype frequencies among mating males (*cf.* CHRISTIANSEN 1980), we have subjected larger numbers of larvae from each progeny to electrophoresis until the father's genotype is determined for *each wild brood* to at least 99% certainty. The design allows (1) study of allelic segregation ratios; (2) direct comparison of genotype frequencies between samples of males viably present and of males succeeding in mating, simply as ordinary binomial samples, without the complication of iterative "gene-counting" estimation procedures (*cf.* OSTERGAARD and CHRISTIANSEN 1981); (3) comparison of fertile *vs.* all viable females; and (4) search for assortative mating.

The estimation methods of Christiansen and associates will still be preferable when only a few offspring per brood are available.

Here, we study male mating success for both the PGI locus and the phosphoglucomutase (PGM, EC 2.7.5.1) locus of two semispecies of Colias, separated both by elevation and by geographic distance. We find major and repeatable differences in male mating success among genotypes of both loci. For PGI, the differences were predictable.

MATERIALS AND METHODS

Routine procedures: Adult males and females of *Colias philodice eriphyle* Edwards were sampled at Gunnison, Colorado (elevation 2350 meters); adult males and females of *C. eurytheme* Boisduval were sampled at Tracy, California (elevation 30 meters). The usual random net-sampling techniques were used, in which the animals were usually captured unawares while feeding at flowers, searching for mates (males) or ovipositing (females) or interacting with one another. Thus, genotypic differences in flight capacity, which might affect catchability during straight line escape flight, had no major effect on the results of sampling.

When brought into the laboratory, females were fed on dilute honey and confined individually for 1–4 days over vetch (Vicia) or clover (Trifolium) to obtain samples of their eggs, up to 100 in number. The resulting larvae were reared, brood by brood, in plastic cups on an artificial diet modified from that of MORTON (1979). Larvae were taken for electrophoresis, most conveniently as third instars, but sometimes as early as late first instar.

Electrophoresis was done as before (WATT 1977, 1983), with adjustment of homogenization volume and/or sample volume loaded depending on larval size. PGM was stained on the same gels

as PGI (since the loci do not overlap in stain pattern) by adding to the PGI stain recipe (WATT 1977) 160–200 μM glucose-1-phosphate and 2 μM glucose-1,6-diphosphate.

All data tabulations and statistical analyses (GOLDSTEIN 1964; SOKAL and ROHLF 1981; ROHLF and SOKAL 1981) were done using interpreted (Microsoft, Inc.) or compiled (Digital Reseach, Inc.) BASIC programs on CompuPro or CCS microcomputers.

Sampling and test design: paternity determination: The central idea is simple: one obtains eggs from each female (if fertile) sampled from the wild and determines her genotype and then the genotype of enough of her progeny to specify her mate's genotype to 99% certainty. The life cycle stage at which the progeny are analyzed must show the same, or at least equally identifiable, phenotype as the adults at the loci studied; this is so for the loci studied here. As soon as two different alleles at a locus are found to come from the father, he is known with certainty to be that particular heterozygote. To assign a homozygous genotype to the father, we require that the chance of a second allele being present but not transmitted by him be reduced below 1%. If Mendelian segregation probabilities of 0.5 for each allele in a heterozygote is assumed, 0.5^n (n = number of progeny run) gives the chance that a male carries two different alleles but only transmits one of them to the progeny sampled. If a female is homozygous, $n = 7$ progeny with the same paternal allele reduce the probability of a second paternal allele's presence to $0.5^7 = 1/128 < 0.01$. If, as is often so for our protocol (see later), eight progeny are run with only one paternal allele seen, $0.5^8 = 1/256$, then the paternity is certain to be better than 99.5%.

If the female is heterozygous, paternity determination for her brood requires more work. Suppose a female has the genotype *ij* and only *ii* and *ij* progeny are seen in her brood. The father can only be *ii* or *ij*. If he were *ij*, the probability that any one offspring is not *jj* = 0.75, so the chance of getting only *ii* and *ij* among n offspring is 0.75^n. $n = 16$ reduces this to 0.01. Furthermore, we cannot know whether a given *j* allele among the *ij* offspring is maternal or paternal in origin, so the proportions of *ii* and *ij* offspring are also relevant. An *ij* × *ii* cross would be unlikely to yield only two *ii* and 14 *ij*. (The exact binomial probability of that or a more extreme segregation from an *ij* mother, given an *ii* father, is only about 0.002, or 0.004 if the other extreme tail, *i.e.*, 14:2 and beyond, is included.) To test for compatibility with *ii*-father or *ij*-father cases, we tabulate all possible partitions of the *i* and *j* alleles between mother and father, given the observed data including the absence of *jj*, and sum the exact binomial probabilities of those partitions. If either case is incompatible with the observed segregation ratio at the 1% level, it is rejected. This is the only possible test if fewer than 16 progeny are able to be genotyped. An analogous set of problems, dealt with in an analogous way, arises from a female *ij* × male *k?* cross, while only *ij*, *ik* and *jk* offspring appear in the progeny.

Because our electrophoresis cells accommodate 40 samples each, we run larvae from various broods in lots of four, examining ten broods per cell per run. If paternity is not determined for a brood in the first run, another block of four larvae is run, and so on until paternity is at least 99% certain for both loci. This often results in the certainty being much greater than 99% for one locus.

Sampling and test design: segregation ratio: Gametic selection (= "meiotic drive") and early zygotic selection on particular allele combinations (or nearby linked loci) could each produce distorted segregation ratios at the loci studied. To the extent that either occurred *in a consistent direction*, it would bias against correctly identifying as fathers male heterozygotes carrying the less often transmitted allele. (This could only be done where clear identification of male and female gametes was possible, *i.e.*, in all progeny for matings of different heterozygotes or of a homozygote and a heterozygote, in homozygotes only for *ij* × *ij* matings.) The resulting $2 \times n$ (n = number of broods) contingency tables, one for each heterozygote in each sample, were then analyzed at two levels for deviation from expected 1:1 segregation and for the nature of such deviation if present.

First, each $2 \times n$ table was analyzed for heterogeneity in goodness of fit to a 1:1 ratio, brood by brood, *via* χ^2 with n-1 d.f. When heterogeneity was found, the one or two most extreme broods were removed and χ^2 was recalculated; insignificance on recalculation identified the heterogeneity as due to the most extreme broods, whereas retention of significance indicated the whole array was heterogeneous. We noted whether the most extreme broods in each table favored the same, or opposite, alleles in their deviations.

Second, the table segregation ratio totals were tested against the 1:1 expectation via χ^2 with 1 d.f. If deviation were found, we again tested whether this was a general effect, or due only to the

most extreme broods, by removing the latter and recalculating χ^2. If segregation bias in a given table was not due only to the most extreme broods, we then asked whether that bias was seen for the same genotype in other samples.

If a particular brood were to show, *a priori*, a distorted paternal segregation ratio, it would require, on average, more larvae to be run before male heterozygosity could be determined than if the segregation ratio were 1:1 as expected. Thus, extreme paternal segregations may bias the total segregation ratio in two ways: by their own distortion of the totals and by the underrepresentation in the totals, compared to the extreme broods, of the more normally segregating broods. Apparent excess transmission of one allele must, therefore, be interpreted very cautiously, unless the effect is general across broods or the same number of larvae were run in all broods compared. Initially suspicious results could be followed up by more elaborate experiments especially to verify whether allele-specific segregation distortion might exist.

PREDICTIONS CONCERNING MATING SUCCESS OF SPECIFIC GENOTYPES

Predictions of differences in fitness indices and components among Colias' PGI genotypes have been made from consideration of biochemical differences among those genotypes in relation to PGI's role in the support of flight and the thermal dependence of flight (WATT 1983). Briefly, as the full argument is extensive, carbohydrate metabolism is the primary source of replenishment of flight muscle ATP pools depleted by flight activity in these insects. Within glycolysis, the first part of this metabolic sequence, PGI is an intervening step among allosterically modifed "control" enzymes. As such, it is selected to maintain a high V_{max}/K_m ratio, thus minimizing its interference either with fast glycolytic response in transient conditions or with maximum throughput capacity in steady-state conditions. Those genotypes with the highest V_{max}/K_m ratios, whether by intrinsic kinetic advantage in low to moderate temperature habitats, by superior thermal stability in high temperature conditions, or some appropriate balance among these characteristics in normally fluctuating thermal habitats, will have superior capacity to sustain flight, especially outside the optimal body temperature range of 35–39°. Thus, they will be favored with respect to all fitness indices and components affected by flight capacity: access to nectar food, finding of mates or oviposition sites, escape from predators or threatening weather conditions, etc.

Although our predictions have so far been semiquantitative and concerned with the *order* of selective discrimination rather than exact quantitative levels, they have been highly successful. Predictions verified in the field (WATT 1977, 1983; WATT, CASSIN and SWAN 1983) include (1) higher survival of kinetically favored heterozygotes in low to moderate habitat temperatures; (2) flight initiation earlier in the day (= greater access to nectar food for both sexes and earlier access to females for males) by kinetically favored heterozygotes; (3) reversal of initially kinetically favored genotypes' survival advantage, in favor of the most thermally stable (for the most part, initially kinetically *dis*favored) genotypes, under unusual heat stress; (4) among the most common genotypes (heterozygote 3/4 and homozygotes 3/3 and 4/4), differences in flight density through the day (= feeding advantage, courtship advantage for males and oviposition advantage for females) occur in the order $3/4 > 3/3 \gg 4/4$.

Sustenance of flight is crucial to mating success for Colias males. Most of their flight time is spent patrolling the top of the vegetation for newly receptive

females (*e.g.*, KINGSOLVER 1983b). Since most new females emerge from their pupae early in the day, males able to be active in the early, cool parts of the day will be most successful at finding them. Thus, kinetically favored male genotypes, especially the heterozygote 3/4, should find a disproportionate percentage of new females. Furthermore, the courtship and remating of older females, which are more selective as to mate choice (*e.g.*, TAYLOR 1972), poses a challenge to the flight vigor and durability of Colias males (*cf.* SILBERGLEID and TAYLOR 1978; RUTOWSKI 1978); this, again, should select for those PGI genotypes most able to sustain glycolytic support of flight.

Thus, we predict that, from flight capacity as fitness index to mating success as fitness component, in low and moderate temperatures, concerning these allozyme genotypes, (1) overall PGI heterozygosity should be higher among those Colias males mating females than among those simply viable and flying with the females; (2) kinetically effective heterozygotes (all except 4/5) should be overrepresented in those males mating, as compared to those flying with, females; (3) the three least kinetically favored genotypes—4/4, 4/5, 5/5—should be least well represented among males successfully mating; (4) the most kinetically disadvantaged common genotype, 4/4, in particular should show poor mating success.

In contrast, we do not expect to see major PGI genotype-specific differences in mating success rates of Colias females, since the mating system is a males-find-and-court, females-choose system (SILBERGLEID and TAYLOR 1978; RUTOWSKI 1978). *Very* small percentages of Colias females fail to be mated at least once in the wild (*e.g.*, GRAHAM, WATT and GALL 1980), except perhaps in very low density populations.

We have as yet no biochemical characterization of Colias' PGM genotypes, so data taken on mating success at this locus constitute initial exploration, not test of predictions.

RESULTS

Segregation patterns at both loci: We found no progenies ($n = 198$) with other than single paternity for each brood, thus further reinforcing the results of BOGGS and WATT (1981) that sperm precedence is absolute in Colias.

Table 1 summarizes segregation ratio data for all common heterozygous allele combinations at both PGI and PGM loci. In many genotypic cases, significant among-broods heterogeneity was seen with respect to the 1:1 segregation expectation. In all but one case, removal of the one or two most extreme broods removed significance, indicating that heterogeneity was not general, but characteristic of a few specific broods. The one exception was female 2/3 PGI heterozygotes on August 8, 1983, wherein two of the three broods showed 3:9 ratios for the maternal alleles; no such effect was seen otherwise for this genotype. In approximately half of the cases, the most extreme broods favored *opposite* alleles.

Only when the two most extreme segregations favored the same allele did the all-brood totals for any genotype in any sample show significant deviation from 1:1 proportions. In those cases, removal of the most extreme broods

removed significant deviation, except for female PGI 2/3 on August 8, 1983 (as before), and for male PGM 3/4 on September 24, 1983. In neither case was the effect seen for that genotype in any other sample.

These data do not show any segregation distortion associated with alleles of the primary loci studied. Since the individually deviant broods as often favor the opposite as the same alleles in particular heterozygote cases, and significant deviations from 1:1 in total are not reproducible within genotypes, the data show the intermittent presence of deleterious or segregation-distorting alleles at other loci in linkage equilibrium with the PGI and PGM loci (disequilibrium would favor one or another of the primary-locus alleles across broods). These effects have had no major biasing effect on paternity determination but in any event would have biased *against* the results found.

Males flying vs. *males mating—both loci*: As Table 2 demonstrates, our predictions concerning male success of PGI genotypes are confirmed in detail.

First, heterozygosity, particularly involving the most kinetically effective heterozygotes, is always significantly greater, up to 50% so, in males successfully mating females than among males flying with those females, in both taxa and in all three samples.

The divergences of mating *vs.* viable males from their own samples' Hardy-Weinberg expectations are equally striking. For *C. p. eriphyle* on August 8, 1983, at Gunnison, males flying were *below* Hardy-Weinberg heterozygosity expectation by 12.5% (a repeat of the temporally based heterozygote deficiency seen previously in this brood of this population; WATT 1983), whereas males mating were *above* expected heterozygosity by 3.3%. For *C. eurytheme* at Tracy, on September 24, 1983, males flying showed 5.4% deficiency in heterozygotes, whereas males mating showed 9.8% excess; on November 3, 1983, in this same population, males flying showed 4.5% deficiency in heterozygotes, whereas males mating showed 14.6% excess! These results are themselves significant by 2×3 contingency table analysis, using the Hardy-Weinberg counts as "expected" data: $G = 7.49$, d.f. $= 2$, $0.01 < P < 0.025$. This test is of course intrinsically less powerful than Goldstein's x* used in Table 2.

The three least kinetically favored genotypes were underrepresented among mating males compared with flying males in all three samples, although this was significant only in the two larger samples. The least kinetically effective homozygote, 4/4, was always significantly deficient among mating males compared with those flying.

These results are especially striking since, like many of our earlier field tests of biochemically and thermal ecologically derived predictions, they were obtained across quite different genetic backgrounds. *C. p. eriphyle* and *C. eurytheme* occupy approximately similar thermal habitats (*e.g.*, WATT, CASSIN and SWAN 1983), are interfertile where they make geographic contact (TAYLOR 1972) and share PGI alleles (WATT 1977) but are nonetheless at least semi-specifically distinct.

As noted before, we have as yet no functional basis for prediction of fitness-related differences among Colias' PGM genotypes. PGM shows *no* coordinate variation with PGI in survivorship, flight duration or other fitness indices/

TABLE 1

Segregation ratios for heterozygous allele combinations at two Colias glycolytic loci

Locus	Species	Sample date	Sex		Genotype 2/3	2/4	3/4	3/5	1/3
PG1	*C.p. eriphyle*								
		August 8, 1983	Male	*N*	5		9	4	
				Table χ^2	11.0(4), $P < 0.05$		17.7(8), $P < 0.025$	5.6(3), $P < 0.5$	
				Extremes	1:6		14:2	7:1	
				Allele totals	26:29		63:52	21:18	
				Total 1:1 $\chi^2(1)$	0.07, $P < 0.9$		0.87, $P < 0.5$	0.10, $P < 0.9$	
		August 8, 1983	Female	*N*	3		16		
				Table χ^2	6.5(2), $P < 0.05$		28.3(15), $P < 0.025$		
				Extremes	3:9, 3:9		11:1		
				Allele totals	9:23		107:80		
				Total 1:1 $\chi^2(1)$	5.3, $P < 0.025$‡		3.6, $P < 0.1$		
PG1	*C. eurytheme*								
		September 24, 1983	Male	*N*	10	5	25		
				Table χ^2	5.8(9), $P < 0.9$	4.1(4), $P < 0.5$	52.7, $P < 0.001$		
				Extremes	4:10	2:5	15:1, 8:1, 1:7		
				Allele totals	46:50	16:28	135:91		
				Total 1:1 $\chi^2(1)$	0.09, $P < 0.9$	2.8, $P < 0.1$	9.0, $P < 0.005$		
		September 24, 1983	Female	*N*	7		24	2	
				Table χ^2	16.2, $P < 0.025$		28.8(23), $P < 0.5$	0.39(1), $P < 0.9$	
				Extremes	1:11		5:1, 1:5	4:3	
				Allele totals	32:58		135:124	11:12	
				Total 1:1 $\chi^2(1)$	6.9, $P < 0.01$		0.38, $P < 0.9$	0.04, $P < 0.9$	
		November 3, 1983	Male	*N*	3	4	18	3	
				Table χ^2	3.9(2), $P < 0.5$	0.09(3), $0.9 < P$	19.2(17), $P < 0.5$	1.3(2), $P < 0.9$	
				Extremes	6:13, 7:4	6:5	7:1, 8:1	4:7	
				Allele totals	16:22	18:17	76:80	11:16	
				Total 1:1 $\chi^2(1)$	0.66, $P < 0.5$	0.03, $P < 0.9$	0.06, $P < 0.9$	0.59, $P < 0.5$	
		November 3, 1983	Female	*N*	5		15	3	
				Table χ^2	2.8(4), $P < 0.9$		16.8(14), $P < 0.5$	0.73, $P < 0.9$	
				Extremes	6:2		8:1, 1:5	5:3	
				Allele totals	27:28		79:75	15:11	
				Total 1:1 $\chi^2(1)$	0.02, $P < 0.9$		0.06, $P < 0.9$	0.35, $P < 0.9$	

PGM	*C.p. eriphyle*						
	August 8, 1983	Male	*N*	8	2	9	3
			Table χ^2	18.0(7), $P < 0.025$	5.6(1), $P < 0.025$	12.1(8), $P < 0.5$	6.9(2), $P < 0.05$
			Extremes	9:1	3:9	8:1, 4:8	2:8
			Allele totals	47:32	9:22	46:34	14:26
			Total 1:1 $\chi^2(1)$	2.5, $P < 0.5$	4.7, $P < 0.5$	1.5, $P < 0.5$	3.0, $P < 0.1$
	August 8, 1983	Female	*N*	10	2	4	
			Table χ^2	20.2(9), $P < 0.025$	0.4(1), $P < 0.9$	19.0(3), $P < 0.005$	
			Extremes	9:1, 0:8		18:2, 8:1	
			Allele totals	54:56	20:20	45:18	
			Total 1:1 $\chi^2(1)$	0.01, $P < 0.975$		10.7, $P < 0.005$	
PGM	*C. eurytheme*						
	September 24, 1983	Male	*N*	12	3	17	2
			Table χ^2	21.3(11), $P < 0.05$	3.4(2), $P < 0.5$	30.6(16), $P < 0.025$	0.3(1), $P < 0.9$
			Extremes	1:8, 2:9, 4:14		7:1, 11:4	
			Allele totals	45:70	20:17	103:59	21:18
			Total 1:1 $\chi^2(1)$	5.0, $P < 0.025$	0.1, $P < 0.9$	11.4, $P < 0.001^{\ddagger}$	0.1, $P < 0.9$
	September 24, 1983	Female	*N*	8		10	
			Table χ^2	6.6(7), $P < 0.5$		29.9(9), $P < 0.005$	
			Extremes	7:1, 4:8		2:17, 0:6	
			Allele totals	54:48		68:50	
			Total 1:1 $\chi^2(1)$	0.4, $P < 0.9$		2.5, $P < 0.5$	
	November 3, 1983	Male	*N*	5	2	13	2
			Table χ^2	2.1(4), $P < 0.9$	4.0(1), $P < 0.05$	19.4(12), $P < 0.1$	1.7(1), $P < 0.5$
			Extremes	2:5	9:3	16:1, 2:6	5:10
			Allele totals	22:29	19:9	69:51	11:16
			Total 1:1 $\chi^2(1)$	0.7, $P < 0.5$	2.9, $P < 0.1$	2.4, $P < 0.5$	0.59, $P < 0.5$
	November 3, 1983	Female	*N*	5		11	
			Table χ^2	7.3(4), $P < 0.5$		14.3(10), $P < 0.5$	
			Extremes	3:11, 6:2		7:1, 6:1	
			Allele totals	25:29		66:44	
			Total 1:1 $\chi^2(1)$	0.17, $P < 0.9$		4.0, $P < 0.05$	

N = number of broods, Table χ^2 = total heterogeneity χ^2 for $2 \times N$ table tesing fit of each brood to 1:1 segregation expectation, as discussed in text. Degrees of freedom (in parentheses) follow each χ^2 value. Extremes are the most extreme segregation ratios seen for each genotype; in approximately half of the cases for each locus, the two or three most extreme broods favor the same allele and in the other half the two different alleles. Total 1:1 χ^2 = value (using Yates' correction for continuity, except where that would produce $\chi^2 = 0$) for test of allele totals, over all broods, to the 1:1 expectation. In all but two cases, marked with ‡, removal of the one or two most extreme broods and recalculation of χ^2 renders deviation from 1:1 expectation insignificant. See text for further interpretation.

TABLE 2

Genotype frequency comparisons between sampled viable males and males successfully mating

Comparison	Sample	Prediction	Males viable		Males mating		x*	*P*
			No.	%	No.	%		
A. PGI								(one tail)
1. All heterozygotes		More among males mating						
	C.p. eriphyle August 8, 1983		32/74	43.2	31/50	62.0	2.05	0.022
	C. eurytheme September 24, 1983		38/78	48.7	58/80	72.5	3.06	0.001
	November 3, 1983		44/92	47.8	45/59	76.3	3.47	<0.001
2. Kinetically favored heterozygotes		More among males mating						
	C.p. eriphyle August 8, 1983		30/74	40.5	29/50	58.0	1.91	0.028
	C. eurytheme September 24, 1983		34/78	43.6	55/80	68.8	3.19	<0.001
	November 3, 1983		42/92	45.7	42/59	71.2	3.0	0.001
3. Three kinetically least favored genotypes		Less among males mating						
	C.p. eriphyle August 8, 1983		13/74	17.6	5/54	9.3	−1.34	0.090
	C. eurytheme September 24, 1983		12/78	15.4	4/89	4.5	−2.39	0.008
	November 3, 1983		13/92	14.1	2/60	3.3	−1.97	0.015

4. 4/4 (kinetically least effective genotype)		Less among males mating						
	C.p. eriphyle							
	August 8, 1983		11/74	14.9	3/54	5.6	−1.67	0.048
	C. eurytheme							
	September 24, 1983		10/78	12.8	2/84	2.2	−2.62	0.004
	November 3, 1983		9/92	9.8	1/60	1.7	−1.97	0.024
B. PGM								(two tails)
All heterozygotes		None						
	C.p. eriphyle							
	August 8, 1983		36/74	48.6	33/52	63.5	1.64	0.11
	C. eurytheme							
	September 24, 1983		29/69	42.0	53/81	65.4	2.87	0.004
	November 3, 1983		44/92	47.8	37/54	68.5	2.43	0.015

x* = Goldstein's (1964) binomial test statistic for difference of percentages, which is approximately a normal deviate and is tested as such. All tests for PGI are one-tailed, as they test specific predictions; PGM tests are two-tailed. Males viable = males caught flying with females in the wild. Males mating = sample of paternities determined from broods of females sampled in the wild.

TABLE 3

Heterozygosity and allele frequency for Colias females in lab paternity testing

Species	Sample date	Female category	PGI				Heterozygosity
			n	*R*	Counts	%	x*
C. p. eriphyle	August 8, 1983	Sampled	65(62)	2.45 ±0.44	33/64	51.6	
	August 8, 1983	Fertile	55	2.39 ±0.39	28/55	50.9	0.07, $P = 0.96$
C. eurytheme	September 24, 1983	Sampled	131(121)	2.85 ±0.70	73/131	55.7	
	September 24, 1983	Fertile	93	2.81 ±0.65	50/93	53.8	0.29, $P = 0.78$
C. eurytheme	November 3, 1983	Sampled	89(83)	3.59 ±0.79	44/89	49.4	
	November 3, 1983	Fertile	70	2.97 ±1.40	35/60	58.3	−1.06, $P = 0.29$

Number of females sampled from the wild is followed in parentheses by the number laying any fertile eggs in the lab. R = wear rating or age index (WATT 1983). "Fertile" females are those producing enough viable larvae to determine paternity for at least one of the loci under study. Goldstein's x* is defined in the caption to Table 2. Tests of allele frequency differences are not tabled, as all are completely insignificant; the *smallest P* value is for PGI p_3 on November 3, 1983, with x* = 0.56 and $P = 0.58$!

components previously studied for Colias' PGI (P. A. CARTER and W. B. WATT, unpublished data). But, we do find, in all three samples, PGM heterozygote excess among mating males as compared with flying males (Table 2), which is significant in the two larger samples. (These data are tested with two tails, unlike those for PGI, since for PGM there is no predicted direction of difference between mating and flying males; this further reduces power of discrimination.)

Female mating patterns and tests for assortative mating: Table 3 presents data on female reproduction as a function of allele and genotype frequencies at both loci. Few females failed to lay at least some fertile eggs in the tests. In no case was there any significant difference in allele frequency or overall heterozygosity, at either locus, between those females caught in the sample and those whose progeny yielded paternity determination, although the fraction of broods viable enough to reveal paternity did vary among the samples. There was a slight but consistent tendency for the oldest females, as indexed by their wear ratings, to lay eggs of lower average viability.

For neither locus was there any significant tendency to assortative mating, as indexed by tendency of all female heterozygotes, or of female kinetically

PGM			Allele frequencies								
			PGI				PGM				
Counts	%	x*	p_2	p_3	p_4	p_5	p_1	p_2	p_3	p_4	p_5
32/63	50.8		0.04	0.56	0.38	0.03	0.02	0.14	0.68	0.15	0.01
		0.09, $P = 0.94$									
27/54	50.4		0.05	0.55	0.37	0.03	0.02	0.15	0.67	0.15	0.01
53/125	42.4		0.08	0.62	0.27	0.03	0.02	0.08	0.77	0.11	0.02
		−0.29, $P = 0.78$									
40/92	44.0		0.07	0.63	0.29	0.22	0.03	0.08	0.77	0.10	0.02
40/87	46.0		0.05	0.64	0.26	0.05	0.01	0.10	0.71	0.17	0.01
		−0.28, $P = 0.78$									
29/60	48.3		0.08	0.61	0.27	0.04	0.01	0.09	0.71	0.19	0.00

favored heterozygotes, to mate either more or less often with their male counterparts than did homozygote females or all females (Table 4).

Search for interaction effects between loci: For each sample of paternities, we tabulated the frequencies of all two-locus, PGI-PGM, genotypes and compared them to the values expected from cross-multiplication of the single-locus genotype frequency vectors. We do not present this large volume of numbers, as no significant deviation from null hypothesis expectations of complete independence of the two loci was found in any sample, for any genotype combination. No consistency in signs of the differences from expectation was seen from sample to sample, and only four differences from expectation as large as ±4% were seen among a total of 182 comparisons over all samples. The statistically significant effects of these genotypes on male-mating success operate entirely without epistatic relations between the loci.

DISCUSSION

Much of empirical population genetics has tried to associate net fitness differences with genotypic alternatives, without attention to the adaptive mechanisms underlying any such differences. This leaves positive results open to the confounding issue of hitchhiking, as noted before. It makes negative results no less ambiguous. For example, YAMAZAKI (1971) found no significant fitness differences among esterase-5 genotypes in a cage study of *Drosophila pseudoobscura*, but since we know nothing of the functional role of this enzyme, nor

TABLE 4

Tests for assortative mating

Species	Sample date	Mating behavior	PGI						PGM		
			All heterozygotes			Kinetically favored heterozygotes			All heterozygotes		
			Counts	%	x*	Counts	%	x*	Counts	%	x*
C. p. eriphyle	August 8, 1983	Males mating all females	31/50	62.0		29/50	58.0		33/52	63.5	
					0.36, $P = 0.72$			0.03, $P = 0.97$			-0.05, $P < 0.61$
	August 8, 1983	Males matching their mates	15/26	57.7		15/26	57.7		18/26	69.2	
C. eurytheme	September 24, 1983	Males mating all females	58/80	72.5		55/80	68.8		53/81	65.4	
					0.43, $P = 0.67$			0.07, $P = 0.95$			-0.54, $P = 0.59$
	September 24, 1983	Males matching their mates	31/45	68.9		30/44	68.2		24/34	70.6	
C. eurytheme	November 3, 1983	Males mating all females	45/59	76.3		42/59	71.2		37/54	68.5	
					0.22, $P = 0.83$			-0.16, $P = 0.87$			-0.20, $P = 0.84$
	November 3, 1983	Males matching their mates	26/35	74.3		24/33	72.7		17/24	70.8	

Goldstein's x* is defined in the caption in Table 2. "Males matching their mates" are, for example, male heterozygote paternities determined on the broods of heterozygous mothers, etc.

of the extent of functional differences among its genotypes, we do not even know whether any realistic opportunity for selection on this locus was present in the experiment.

By contrast, adaptive studies beginning with the functional differences among natural polymorphs can lead to greater clarity of final results, although in initial stages their results may appear remote from direct connection to fitness differences. Thus, in the snail Cepaea, study of predator-prey interactions and thermal ecology have alike given insight into some evolutionary causes of genetic polymorphism (JONES, LEITH and RAWLINGS 1977). Other aspects, such as the notorious "area effects," remain controversial (*e.g.*, SELANDER and OCHMAN 1983), very possibly in part because of a lack of mechanistic understanding. It has been argued (GRAHAM, WATT and GALL 1980) that beginning the evolutionary study of variation with focus on the variation's mechanism of action, and the identification of possible selection pressures, then following with study of the overall selection regime (broad sense, including the possibility of neutrality), finishing with net fitness estimation, may often be necessary, let alone more practical.

The study of Colias' PGI polymorphism is now approaching its final stages in these terms. To our earlier successful predictions of differences in fitness indices such as flight time through the day and fitness components such as survivorship and female fecundity (egg output = flight time for a mated female; WATT 1977, 1983; STANTON 1980, 1982; KINGSOLVER 1983b; WATT, CASSIN and SWAN 1983), we here add another fitness component difference: male mating success *resulting from* genotypic differences in male flight time and/or capacity. Computer simulation of the selection regime resulting from the interaction of these fitness components now seems a feasible tool for exploring the adequacy of present understanding, for searching out critical new combinations of conditions for field test and for final prediction of *net* fitness differences among genotypes for test in the field or in outdoor population cages.

The present results for PGI were obtained in moderate temperature conditions. Although the V_{max}/K_m ratio differences among genotypes, leading to our predictions, are significant and large at thermally optimum temperatures, they are even more so at suboptimal temperatures (WATT 1983). Thus, we expect to find even more dramatic differences in mating success under lower average temperature conditions—in spring broods, alpine habitats, etc.

By contrast, our study of PGM is just beginning. Our finding of PGM heterozygote advantage in male mating is all the more striking by its independence of PGI. Although PGI and PGM share a common substrate, glucose-6-phosphate, and operate together in all major insect tissues, they nonetheless occupy distinct roles in metabolism. PGM stands athwart the route to and from storage of carbohydrate reserves in glycogen. PGI, although participating in the routing of Colias' dietary fructose (WATT, HOCH and MILLS 1974) to glycogen storage or trehalose synthesis (WATT 1977; C. L. BOGGS and W. B. WATT, unpublished data), is much more concerned with acute glycolytic energy supply in support of flight (WATT 1977, 1983). Genetic variation in PGM of

Tribolium beetles responds to larval diet shifts (RIDDLE, IVERSON and DAWSON 1983); other data on Colias hints at preadult metabolic effects of PGM genotypes (P. A. CARTER and W. B. WATT, unpublished data). RUTOWSKI (1979) finds that males of the genus Pieris, closely related to Colias, court females with an intensity proportional to the resources those males can donate to females during mating. Colias males also donate resources to females during mating (BOGGS and WATT 1981); perhaps those males heterozygous for PGM emerge with more resources carried over from preadult metabolism and, thus, court females more intensely on average than do homozygotes.

Conventional views of evolutionary genetics, whether neutralist or selectionist in orientation, have regarded allozyme variation as likely to be equivalent across loci. Large evolutionary effects at specific loci, or major differences in size of such effects among loci, are alike unexpected by these views. In contrast, mechanistically oriented views expect both of these things. The "bioenergetic" viewpoint (WATT 1985), in particular, holds that the evolutionary impact of variation at a specific locus should be scaled jointly by the nature and size of differences among the genotypes at that locus, by the placement of that enzyme locus in the architecture of its metabolic pathway and by the fraction of the organism's overall energy budget for which that particular pathway is responsible. This view not only accounts for many of the now numerous cases in which functional study of allozyme variants leads to successful explanation or prediction of fitness index/component differences among those variants but can successfully predict when variants at a given locus in different organisms or under different metabolic conditions will and will not be subject to selection (WATT 1984, 1985). Our present results, further confirming specific bioenergetic predictions with respect to Colias PGI, point to the necessity for revision of the expectation that evolutionary forces will act uniformly on diverse gene loci.

We thank CAROL BOGGS, GEORGE CHOW, FREDDY CHRISTIANSEN, MARC FELDMAN, DAVE PERKINS and JON ROUGHGARDEN for stimulating discussion and/or for helpful commentary on this manuscript; STEVE HEWITT, MATT RANDAZZO, JILL RICKETTS and MARY SWAN for superior technical assistance; and RALPH and LOIS WATT, the National Institutes of Health (GM 26758) and the National Science Foundation (DEB 80-11129) for research support.

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Communicating editor: J. R. POWELL