Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 10608–10612, November 1992
Evolution

Eggs, enzymes, and evolution: Natural genetic variants change insect fecundity

(allozymes/fitness components/global warming/phosphoglucose isomerase/thermal ecology)

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Communicated by Paul R. Ehrlich, July 20, 1992 (received for review May 13, 1992)

ABSTRACT **Phosphoglucose isomerase genotypes in the butterfly *Colias* differ dramatically in biochemical properties. These differences were evaluated earlier, using metabolic network theory, to predict, successfully, their effects on glycolytic metabolism and hence on *Colias* flight capacity and several consequent fitness components in the wild. Female egg-laying, not previously studied, also depends on flight, so female fecundity is now predicted to differ among these genotypes. An experimental design incorporating the thermal ecology of *Colias* confirms these predictions in a cool habitat. Thus female fecundity differences among animal enzyme polymorphs have now been found. Quantitative reconstruction of the selection regime for phosphoglucose isomerase genotypes in *Colias* can now begin. The most heat-stable genotypes are the least fecund, suggesting that global warming, if it occurs, may have severe impacts, through population genetics, on demography of thermally sensitive creatures.**

A small but growing body of evolutionary work shows that one can study natural selection (or its absence) experimentally, often by the integrated use of biochemical or physiological methods as well as ecological and genetical ones (1–4). Natural variation at the phosphoglucose isomerase (PGI; EC 5.3.1.9) gene, *Pgi*, of *Colias* butterflies is being studied in this way (5–10). From study of functional differences among the *Pgi* genotypes and of their metabolic and physiological–ecological context, genotypic differences in flight performance and in resulting fitness components—survivorship and male mating success—have been predicted and then found in the wild (5–10). I now report predictable differences among *Colias Pgi* genotypes in female fecundity.

First, let us review the metabolic context for these genotypes' function. Many believe that only "control" or "rate-limiting" enzyme-catalyzed reaction steps are strongly selected for refinement of function. Theory of metabolic networks shows otherwise: intervening steps, such as the reaction catalyzed by PGI, and control steps are interdependent in function (11–14), and variation in both types may have evolutionary consequences (1, 2). The key parameter for each step in an unsaturated metabolic pathway is the V_{max}/K_m ratio, the "pseudo-first-order rate constant" which governs the rate of an enzymic reaction step in the limit of decreasing substrate concentration (1–3, 6). V_{max}/K_m values for all steps in a metabolic pathway jointly determine *in vivo* pathway performance, whether in optimizing control of steady-state flux or in minimizing time for transient response to changed metabolic demand (11–14). The components of V_{max}/K_m are K_m, a composite constant numerically equal to substrate concentration giving half-maximal rate, hence an index of substrate binding; and V_{max}, maximal rate, the product of k_{cat}, the catalytic rate constant, and [E], the enzyme concentration. V_{max}/K_m can change through structural-gene change in k_{cat} and K_m or in [E] due to stability of the enzyme, and also through change in [E] due to genetic variation in transcription or translation.

Sugar "fuel" use by glycolysis and oxidative metabolism, to make ATP in insect flight muscle, is a transient-state metabolic system; the sugar processing rate may increase by 100-fold in the first seconds of flight (15). Colias PGI, catalyzing an intervening step in glycolysis, is selected to maximize its V_{max}/K_m ratio, minimizing the lag in its response to changed metabolic demand (1, 5). This favors high k_{cat} and low K_m (1, 3, 5). (K_m values which are too low compared to substrate concentrations may impair kinetic response (16), but this does not apply here.) Heat stress favors genotypes producing thermally stable PGI enzyme; such genotypes, under such stress, maintain PGI concentration [E] near levels initially synthesized (1, 5).

The thermal ecology of *Colias* constrains its flight, hence its energy demand. *Colias* has a narrow body temperature (T_b) optimum, 35–39°C, for flight (17). Yet, since *Colias* thermoregulates behaviorally, it must often be active at suboptimal T_b, 29–35°C, or withstand high T_b stress, >40°C (17). This exacerbates demands on the response of PGI to rapid changes in glycolytic function, hence on its V_{max}/K_m ratio, as flight starts and stops (1, 4–8); PGIs of low V_{max}/K_m can sustain flight less well at nonoptimal T_b than those of high V_{max}/K_m (7). Thus, thermal variation within and among days (*i*) changes the fraction of the day in which flight can occur and (*ii*) changes thermal stress on the function of PGIs in support of glycolytic fuel supply to flight (5, 6).

Prediction of Genotypic Differences in Female Fecundity

Pgi genotypes' differences in metabolic function produce differences in their flight capacity (7). Egg output varies directly with flight (17–21), so genotypes should differ in egg output. Among frequent-to-common genotypes, 2/2, 2/3, 2/4, 2/5, 3/4, and 3/5 are kinetically favored; of these, 2/2 and 2/4 are very labile to high temperature, and 2/3 is moderately so (5, 6). Genotype 3/3 is of moderate kinetic effectiveness and thermal stability; 4/4, 4/5, and 5/5 are more thermally stable, but less kinetically effective, than the others (5, 6). (This tradeoff of kinetics vs. stability may arise from protein-structure constraints, but structural studies will be needed to decide; cf. refs. 5, 6, and 16.) Thus, since good kinetics yield high flight capacity, unless heat stress reduces the [E] part of V_{max}/K_m in labile genotypes, predictions for cool habitats are that 4/4, 4/5, and 5/5 should have low, 3/3 moderate, and the others high egg yields. Among the three

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Abbreviations: ANOVA, analysis of variance; FAT, flight activity time; GLM, general linear model; PGI, phosphoglucose isomerase.

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most common genotypes, the order of oviposition performance should be 3/4 > 3/3 >> 4/4.

Experimental Assay for *Colias* Female Fecundity

Female *Colias* must fly, after each egg is laid on a larval host plant, to find a new egg-laying site (18–20). To sort out genotypic effects on flight-dependent oviposition from general thermal effects, changes in T_b-dependent flight must be tracked through each day. We monitor thermal variables—solar flux S, air temperature T_a, etc. (Fig. 1)—in the study habitat. From these data and *Colias* thermal parameters (Fig. 1), we compute flight activity time (FAT), using a model of

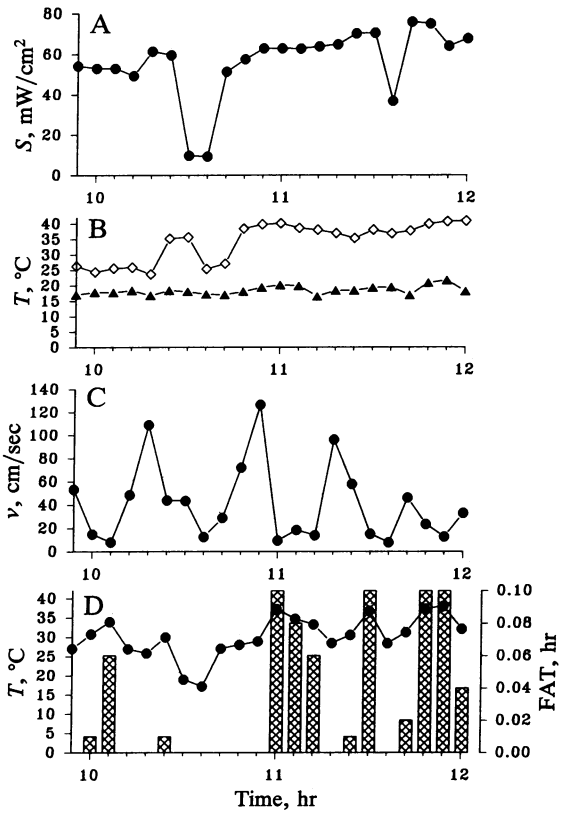

FIG. 1. Example of input to and output from the FORTRAN-77 program TBAL, which calculates *Colias* T_b and FAT. Input data, taken in an oviposition test cage each 0.1 hr by a data logger, are solar flux S, wind speed v, air temperature T_a, ground temperature T_g, and cage wire temperature T_w. S was measured with a LiCor 200B pyranometer, v with a hot-wire anemometer, and T_a, T_g, and T_w with thermocouples. Absorptivity, α, was measured on wings of nine young female *Colias* from the study population over the solar spectrum wavelength range with a Zeiss spectrophotometer; wavelength-specific absorptions were weighted by the solar spectrum to give α for overall S. Insulating fur thickness, δ, was measured on the same insects at the ventral mesothoracic midline with a microscope and ocular micrometer. Means and standard deviations were for α, 0.698 ± 0.028, and for δ, 1.39 ± 0.13 mm. Mean characteristic dimension D (22, 23) = 0.28 cm, and length L = 1.8 cm, with solar noon time, latitude, and longitude (to calculate solar zenith angles), and the other data, are used to solve the *Colias* thermal balance model for T_b at each time point, for lateral-basking and heat-avoiding body orientations to S. T_b values are referred to probability-of-flight vs. T_b data (17) to calculate FAT in each 0.1 hr; FAT values are summed over the day. Data are for 09:54 to 12:00, 09/02/89, at Gothic, CO. (*A*) S. (*B*) T_a (▲) and T_g (◇); T_w is omitted, as it tracks T_a and v closely, ranging from (T_a + 0.5°C) to (T_a + 3°C) in sunlight. (*C*) v, 10 cm above ground. (*D*) Output female *Colias* basking T_b (●) and FAT (bars). Note reduction of T_b below flight-permissive values by cloud interruption of S and by increase in cooling v. See Table 1 for whole-study summary of these results.

Colias thermal balance whose ability to predict temporal boundaries of *Colias* daily flight in the wild has been validated (21–25). Here, FAT indexes thermally constrained opportunity for females to fly, thus to lay eggs (17, 21). How *Pgi* genotypes' flight-dependent egg output varies within this constraint can then be asked separately from assessment of the constraint itself.

Colias fecundity declines with age (26), so this effect must be sorted out from those of genotype or thermal ecology. We do so by rating females, at capture, on a scale of wear damage to their wings (27, 28), ranging in steps of 0.5 from 1.0 (freshly eclosed, wings wet, undamaged) to 5.0 (severely eroded scales and torn wing cuticle).

Normal oviposition by *Colias* females occurs in 0.25-m^3 wire cages (¼-inch mesh, black so reflections do not confuse insects' vision) containing the host plant, grass on which females may rest without chemotactile egglaying stimulation, and an artificial flower for feeding (19). In such cages, outdoors, females undergo daily variation in S, T_a, etc. which, "filtered" by thermoregulation, yields high or low T_b and thus flight or inactivity (17, 21–25). We use such cages, recording microclimate inside one, to relate females' egg outputs to their ages, *Pgi* genotypes, and FAT. Initial work showed that, in a spatially uniform habitat, intercage microclimate variation is negligible.

This assay gives a minimum estimate of fecundity differences in the wild. In the cage, a female, if she can fly at all, can find a host plant quickly once searching begins. In the field, sparse plant distributions may impose long search for an oviposition site, straining flight capacity and making genotypic differences in fecundity even stronger.

Experimental Conditions and Genotypes Studied

Female *Colias philodice eriphyle* Edwards were captured in the wild at Gunnison, CO (elevation 2350 m); ages (= wear values, above) were estimated at capture. Up to 40 females each day were put singly into cages, using as host plant three to six seedlings of a cultivar of *Vicia* (vetch), a native host plant (20). Caged females were put outdoors at Gothic, CO (elevation 2925 m), each morning before thermal conditions allowed T_b to reach 29°C, the base of the voluntary flight range. They were left out (except during rain) until sundown, then stored inactive overnight at 15°C. Next morning, eggs were removed and counted, artificial feeders were refilled, and fresh *Vicia* were provided.

The cages were a cool environment for females. The average female spent only a little time with T_b as high as the 35–39°C optimum (Table 1). Only twice in the study did the computer model show insect overheating; the cage wire reduced solar flux by 30%, though it also reduced wind speed, hence convective cooling, and raised radiative environment temperature. Mean calculated daily FAT in the study was 2.72 ± 1.24 hr (mean ± standard deviation; when grouped categorically for analysis as below, 2.27 ± 1.32 hr); typical values for uncaged individuals of this species would be twice that or more (24).

Each female was studied for 4, sometimes 5 (if little FAT accumulated in the first 4), days unless it died sooner (Table 3). Females were genotyped for *Pgi* as usual (29). In August–September 1989 and August 1990, 255 females were studied. Four *Pgi* genotypes—1/3, 2/2, 2/6, and 4/6—occurred once each, so their data were not analyzed. Seven genotypes were studied over 899 total insect-days; their identities, numbers, and means ± standard errors of means for FAT and wear (= age; refs. 27 and 28) were as follows: 2/3, 16, 2.40 ± 0.18 hr, 2.58 ± 0.09; 2/4, 5, 2.29 ± 0.31 hr, 2.53 ± 0.11; 3/3, 105, 2.31 ± 0.07 hr, 2.59 ± 0.03; 3/4, 73, 2.13 ± 0.08 hr, 2.51 ± 0.03; 3/5, 9, 2.39 ± 0.25 hr, 2.45 ± 0.08; 4/4, 34, 2.23 ± 0.12 hr, 2.67 ± 0.05; 4/5, 9, 2.73 ± 0.26 hr, 2.79 ± 0.14.

Table 1. Summary of daily T_b and FAT calculations from recorded microclimate data throughout the study

Date	Total hr obs	Voluntary flight 29°C < T_b < 40°C: hr obs	Frac	$\bar{T}_b$, °C	Optimal flight 35°C < T_b < 39°C: hr obs	Frac	$\bar{T}_b$, °C	FAT, hr
08/23/89	7.1	2.2	0.31	33.3	0.6	0.09	35.6	1.12
08/24/89	8.3	3.5	0.42	31.8	0.3	0.04	35.8	1.19
08/25/89	8.3	3.9	0.47	32.9	0.7	0.08	37.0	1.84
08/26/89	7.9	5.8	0.73	33.6	1.9	0.24	36.3	3.31
08/27/89	8.2	4.3	0.52	32.9	0.9	0.11	36.1	2.04
08/28/89	8.2	5.5	0.67	35.8	3.7	0.45	36.7	4.42
08/29/89	8.1	5.7	0.70	33.7	1.8	0.22	36.6	3.33
08/30/89	5.9	1.0	0.17	33.3	0.4	0.07	36.7	0.53
08/31/89	7.7	5.8	0.75	33.7	1.9	0.25	36.2	3.41
09/01/89	7.9	6.3	0.80	34.1	2.6	0.33	36.6	3.99
09/02/89	7.4	5.6	0.76	33.5	2.1	0.28	36.1	3.18
09/03/89	7.6	5.7	0.75	34.2	2.3	0.30	35.9	3.36
09/04/89	7.2	4.2	0.58	34.8	1.5	0.21	36.5	2.73
09/05/89	8.0	5.6	0.70	34.4	2.3	0.29	36.5	3.91
08/05/90	7.9	5.2	0.66	35.9	3.7	0.47	36.1	4.51
08/06/90	7.0	4.3	0.61	33.5	1.3	0.19	36.7	2.22
08/07/90	7.8	7.0	0.90	35.9	4.7	0.60	37.0	5.52
08/08/90	4.4	3.0	0.68	34.4	1.4	0.32	36.8	1.94
08/10/90	3.1	2.1	0.68	35.7	1.3	0.42	37.6	1.67
08/11/90	6.1	4.7	0.77	33.7	1.6	0.26	36.5	2.77
08/12/90	5.6	1.7	0.30	34.6	0.7	0.13	36.2	1.31
08/13/90	2.9	0.7	0.24	32.0	0.1	0.03	35.1	0.26
08/14/90	6.3	0.4	0.06	32.6	0.2	0.03	35.4	0.22
08/19/90	5.0	1.9	0.38	31.7	0.2	0.04	36.0	0.59
08/20/90	7.1	2.7	0.38	34.9	1.4	0.20	36.1	1.95
08/21/90	7.5	3.1	0.41	34.1	1.4	0.19	36.5	1.90
08/22/90	4.1	0.4	0.10	31.9	0.0	0.00	—	0.14
08/24/90	8.1	2.9	0.36	34.1	1.3	0.16	36.4	1.88
08/25/90	7.9	4.7	0.60	33.1	1.0	0.13	36.4	2.41
08/26/90	7.7	5.7	0.74	33.0	1.7	0.22	36.3	2.83
08/27/90	7.2	5.9	0.82	34.9	2.6	0.36	36.8	3.78

hr obs, hours observed each day, whether total or time spent in a particular T_b range; Frac, fraction of total time spent in a particular T_b range; $\bar{T}_b$, mean T_b within a given T_b range.

Test for Correlation of *Pgi* Genotypes with Thermoregulatory Phenotypes

This study would be confounded if *Pgi* genotypes were correlated with variation in the solar absorptivity or fur (modified scales) thickness of *Colias*. There is no reason to expect phenotypic correlation: glycolysis *per se* is not closely connected, metabolically, to the pigment systems (pteridines or melanin) affecting absorptivity or to fur development. Despite this, and lack of evidence for genetic correlation (cf. ref. 4), the question was tested explicitly. A separate sample of 73 adults was taken from the study population, measured for absorptivity and fur thickness, and genotyped for *Pgi* (all three characters were scored "blind"). As Table 2 shows, neither absorptivity nor fur thickness varied significantly with genotype, and our experimental design is well justified.

Test of All Predictions About Fecundity

Daily egg counts C, as integer data including zero values, were transformed to $(C + 0.5)^{1/2}$ to normalize them (30). In analysis of variance (ANOVA) or general linear model (GLM) analysis, genotype was a category variable; calculated FAT values were grouped into categories: 1, FAT $\leq$ 1 hr; 2, 1 < FAT $\leq$ 2 hr; 3, 2 < FAT $\leq$ 3 hr; 4, 3 < FAT $\leq$ 4 hr; 5, 4 < FAT < 6 hr. Wear was treated as a continuous variable. Initial ANOVAs, tests of count data, or tests of homogeneity of slope of eggs vs. wear (= age) among genotypes and FAT classes were done as usual (30–32). Final ANOVA and GLM analyses used SYSTAT (33). Oviposition rates were compared among specific genotypes with *post hoc* contrasts (30, 32, 33), though some tests were of *a priori* predictions.

Initial analyses showed that (*i*) genotype, FAT, and age (= wear) all had highly significant effects, of the kinds predicted, on egg output; (*ii*) genotype did not interact with age (= wear) in determining egg output (homogeneity of slope of eggs-on-wear regressions among genotypes: $F_{6, 886} = 0.67$, $P = 0.68$); (*iii*) genotype and FAT did not interact in determining egg output (genotype × FAT category interaction insignificant; $F_{24, 859} = 0.85$, $P = 0.68$); (*iv*) age (wear) and FAT did interact significantly in determining egg output. Table 3 presents the final linear model: $(C + 0.5)^{1/2}$ = constant + *Pgi* genotype + FAT + age + FAT × age. It also presents specific genotypic "contrasts."

Egg output dropped with female age and increased with daily FAT, agreeing with earlier work (17–21, 26) and our predictions. These variables interacted: at FAT < 1 hr no insect laid eggs very well, but older insects were much less able than young ones to increase egg-laying as FAT increased. Both main effects and this interaction were significant (Table 2). The genotypes were homogeneous for experience of daily FAT by ANOVA ($F_{6, 892} = 1.53$, $P = 0.17$), and also for initial wear (= age) by ANOVA ($F_{6, 244} = 0.78$, $P = 0.59$).

Fig. 2*A* presents numbers of females laying eggs. While about 60% of all females actually laid eggs in the study, significantly more 3/4 females (68%) did so than either 3/3 females (57%) or 4/4 females (44%). Fig. 2*B* and Table 3 show that the genotypes differed sharply in egg yield per day. Among the most common genotypes, the egg yield order was, as predicted, 3/4 > 3/3 >> 4/4, with $P < 0.001$. Again as predicted, with $P < 0.001$, the kinetically disfavored genotypes 4/4 and 4/5 had the lowest egg outputs. Genotype 2/3 laid fewer eggs than expected, but contrast analysis (Table 3) shows that it was not significantly different from other kinetically moderate or good genotypes. More work is needed on this point. All other kinetically favored genotypes had high egg output as predicted. The lack of genotype × FAT interaction makes sense, as all females were exposed mainly to suboptimal thermal conditions. A test habitat allowing more FAT per day, including a higher fraction of optimal T_b, might show interaction, as genotypes disadvantaged at low T_b might improve performance disproportionately at optimal T_b.

Implications for Fundamental and Applied Evolutionary Biology

Throughout our study of *Colias Pgi* polymorphism and its functional context, two levels of null hypothesis have been considered. The "simple neutral" view is that genotypes do not differ in function. This has been rejected at all levels of function studied (5–10), and here as well. The more subtle "associative neutral" view is that differences result from "hitchhiking" (34)—linkage disequilibrium between *Pgi* and unknown genes. This is also rejected for *Colias Pgi*, as now reviewed (cf. also ref. 1).

Hitchhiking is very vulnerable to recombination (34–37). Its main effect now seems to be reduction of neutral variation around directionally selected allelic sites by rapid "selective sweeps," before recombination can destroy disequilibrium between the new favored allele and neutral variants (38, 39). In *Drosophila*, the zone in which neutral variants hitchhike with a polymorphic selected site is only a few hundred base

Table 2. Statistical analysis of test for association of *Pgi* genotype with thermoregulatory parameters of *Colias*

	Genotypes								Sexes			
	3/3		3/4		4/4		Other		Male		Female	
Phenotype	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$	n	$\mu \pm \sigma$
α	28	0.653 ± 0.055	31	0.648 ± 0.042	6	0.658 ± 0.034	8	0.683 ± 0.050	49	0.642 ± 0.050	24	0.678 ± 0.034
δ	28	0.86 ± 0.10	31	0.87 ± 0.12	6	0.85 ± 0.07	8	0.82 ± 0.15	49	0.83 ± 0.10	24	0.87 ± 0.13

Multiway ANOVA

	α					δ				
Source	SS	df	MS	F	P	SS	df	MS	F	P
Genotype	0.0070	3	0.0023	1.0864	0.361	0.0132	3	0.0044	0.3313	0.803
Sex	0.0130	1	0.0130	6.1005	0.016	0.0111	1	0.0111	0.8316	0.365
Interaction	0.0006	3	0.0002	0.0866	0.967	0.0267	3	0.0089	0.6692	0.574
Error	0.1388	65	0.0021			0.8644	65	0.0133		
Total	0.1594	72				0.9154	72			

Numbers (n), cell means (μ) ± standard deviations (σ), and multiway analysis of variance (ANOVA; SYSTAT 5.0) of absorptivity (α, units 0.0–1.0) and fur thickness (δ in mm) vs. *Pgi* genotype and sex in *C. philodice eriphyle*. SS, sum of squares; MS, mean square; df, degrees of freedom; F, F-ratio; P, probability of finding test statistic value by chance alone (30). The three most common genotypes—3/3, 3/4, and 4/4—were analyzed as separate groups, and other genotypes were pooled as a fourth group, under genotype. *Pgi* genotype does not affect either of the thermoregulatory parameters. See text for further details.

pairs wide (40), well within the gene containing the selected site. *Drosophila* has a lower recombination rate r—1.58 centimorgans (cM) per million DNA base pairs (Mbp), or 285 cM per genome—than the *Colias* relative, the moth *Bombyx*—5.8 cM/Mbp, or 2900 cM per genome (41). The r of *Colias* seems like that of *Bombyx*: e.g., genes for phosphoglucomutase and glucose-6-phosphate dehydrogenase are 41.5 cM apart (29) on one of the 31 similar-sized haploid chromosomes of *Colias* (42), implying $r \geq 1300$ cM per genome. Thus the zone of neutral hitchhiking with a selected site should be even narrower in *Colias* than in *Drosophila*.

Table 3. Summary statistical analysis of *Colias Pgi* genotypes' oviposition

Source	SS	df	MS	F	P
Genotype	151.73	6	25.29	4.32	0.000263
FAT	202.19	4	50.55	8.64	0.000001
Age (wear)	75.56	1	75.56	12.91	0.000345
FAT × age	96.48	4	24.12	4.12	0.002580
Residual error	5167.45	883	5.85		
Contrasts					
3/4 > 3/3 >> 4/4 4/4, 4/5 < all other genotypes	79.51	1	79.51	13.59	0.000242
2/3 ≠ 3/3, 2/4, 3/4, 3/5	71.05	1	71.05	12.14	0.000517
2/3 ≠ 2/4, 3/4, 3/5	13.58	1	13.58	2.32	0.128
	17.19	1	17.19	2.94	0.087

GLM analysis of *Colias* oviposition per day (Fig. 2*B*) vs. *Pgi* genotype, FAT, and age (indexed as wear), the interaction FAT × wear, and genotypic subset comparisons treated as "*post hoc*" contrasts (30, 33). Two other interactions were insignificant in initial analysis and thus are omitted (see text). Given that females have different numbers of days' data, a statistical design nesting individuals, with their daily values of egg count and FAT, under genotypes would be best. Since common genotypes had up to 105 females (Fig. 2*A*), this would have created so many cells in the GLM as to be computationally impractical. Treating each oviposition-day as an independent datum seemed the best statistical compromise. To check for resulting bias, a more summary GLM analysis was run, in which the (square-root-transformed) total eggs from each female was the dependent variable instead of daily egg counts, and total FAT for each female replaced daily FAT values. This decreased the precision of analysis, changing values for SS terms, F ratios, and P values. However, the pattern of significance or its absence was repeated exactly for main effects, interactions, and *post hoc* contrasts. Clearly the more thorough analysis was not, in practice, biased by using multiple daily egg counts from each female.

Flight and fitness component differences among *Colias Pgi* genotypes repeat among populations up to 1900 km apart (5–10). Stable hitchhiking of *Pgi* with unrelated genes, given high r, across such separation is extremely unlikely.

The specificity of prediction of fitness-related differences among these *Pgi* genotypes from their biochemical properties (5–10) is equally damaging to the associative hypothesis. One might explain nonspecific heterosis among allozymes, of unknown mechanism, by association. But to propose without evidence that, e.g., the observed order of performance in flight and fitness components among genotypes 3/4 > 3/3 >> 4/4, which was predicted *de novo* from their metabolic functional differences, is due not to these known differences

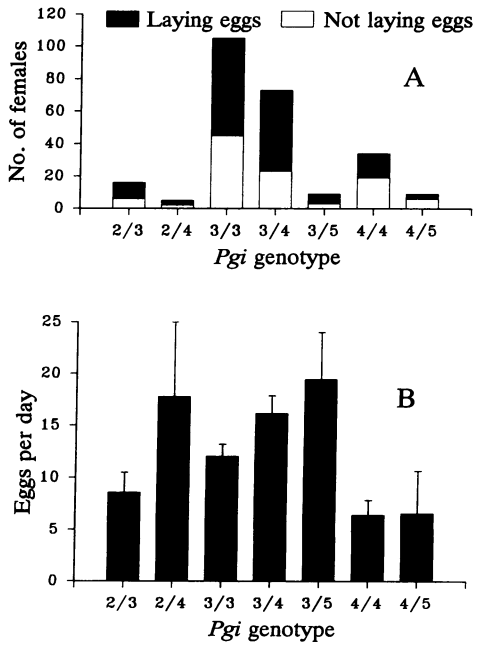

FIG. 2. Daily egg output vs. *Colias Pgi* genotype. (*A*) Females laying or not laying eggs in study. The average laying eggs is roughly 60%, but by Goldstein's x^* test (31), 3/4 (50/73) has more females laying than 3/3 (60/105; $x^* = 1.55$, one-tailed $P < 0.05$) or than 4/4 (15/34; $x^* = 2.40$, one-tailed $P = 0.008$); this order of the genotypes' performance is predicted *a priori* (see text), hence the one-tailed tests. (*B*) Mean daily egg output, untransformed, and standard errors of means. See Table 3 for statistical analysis.

but to variants at linked genes of unknown function, is to violate Ockham's Razor: "Entities are not to be unnecessarily multiplied."

We thus have found here fecundity effects of allozymes in animals. We see predicted differences among *Colias Pgi* genotypes in each major fitness component: survivorship, male mating success, and female fecundity. These differences arise from the genotypes' function, the enzyme's role in a demanding central metabolic system, and the performance needs imposed by the ecology of *Colias* (1, 2). Thermally based selection balances kinetic advantage at low to moderate temperature against episodic heat stress, which favors stable, but kinetically poorer, alternative genotypes (5–10). We can now begin quantitative reconstruction of the whole selective regime for *Pgi* in the lowland species complex of *Colias*.

This work sets up comparative study of other polymorphisms which differ in function. Two other variable enzymes in *Colias*, which share PGI's substrate but differ in metabolic role, do not show flight or survivorship differences among genotypes as does PGI, but do show independent male mating success differences (29). A broad range of selection on gene loci, extending down to "polygene"-sized effects and finally to neutrality (43–45), is to be expected. Where variable genes of diverse creatures fall in this range, in relation to genotypic functional differences, metabolic roles, and centrality to energy budgets (1, 2), should teach us much about the evolution of metabolism. This is an important exemplar for integration of mechanistic and population views of evolutionary problems, as called for by physiologists and evolutionists alike (3, 46–48).

Our data suggest that global warming, a serious hazard (and problem source for applied evolutionary biology) if it occurs, might have population-genetic effects of unforeseen demographic impact. *Pgi* genotypes (4/4 and 4/5 here) which are most stable to high T_b have low fecundity, like their low values of other fitness components, in cool habitats (5–10). But they would disproportionately survive increased heat stress, at $T_b > 40°C$ (6, 7, 17), which would arise with higher mean T_a under global warming. It is unclear if thermal increase of daily FAT, as egg-laying time, could balance low egg-laying rate over that time. *Colias* do not oviposit at hyperoptimal temperatures, flying only to reach shade when $T_b \geq 40°C$ (17), so habitat warming would be unlikely to create a "new environment" in which heat-stable *Pgi* genotypes might perform better. Fecundity is a "key factor" for population size in *Colias* (18). Thus, rise in frequency of stable but unfecund genotypes might sharply reduce population sizes, increasing extinction chances. If new work confirms this, it may be a model for unexpectedly severe global warming impact on thermally sensitive organisms.

I thank C. Boggs, P. Carter, K. Donohue, M. Feldman, D. Gordon, J. Harte, M. Jacobs, E. Leigh, M. Nielsen, D. Perkins, D. Pollock, P. Schulte, and P. Vitousek for discussions; A. Baldo, C. Burge, R. Fenerty, S. Hill, D. Irwin, M. Nielsen, D. Rosenblum, E. Shortwell, and A. Townsend for assistance; R. James Pike for access to his land; and the U.S. National Science Foundation (BSR 87-05268) and National Institutes of Health (GM 26758) for research support.

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