Evolving resistance to obesity in an insect

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Failure to adapt to a changing nutritional environment comes at a cost, as evidenced by the modern human obesity crisis. Consumption of energy-rich diets can lead to obesity and is associated with deleterious consequences not only in humans but also in many other animals, including insects. The question thus arises whether animals restricted over multiple generations to high-energy diets can evolve mechanisms to limit the deposition of adverse levels of body fat. We show that *Plutella xylostella* caterpillars reared for multiple generations on carbohydrate-rich foods (either a chemically defined artificial diet or a high-starch *Arabidopsis* mutant) progressively developed the ability to eat excess carbohydrate without laying it down as fat, providing strong evidence that excess fat storage has a fitness cost. In contrast, caterpillars reared in carbohydrate-scarce environments (a chemically defined artificial diet or a low-starch *Arabidopsis* mutant) had a greater propensity to store ingested carbohydrate as fat. Additionally, insects reared on the low-starch *Arabidopsis* mutant evolved a preference for laying their eggs on this plant, whereas those selected on the high-starch *Arabidopsis* mutant showed no preference. Our results provide an experimental example of metabolic adaptation in the face of changes in the nutritional environment and suggest that changes in plant macronutrient profiles may promote host-associated population divergence.

geometric framework | nutrition | *Plutella*

A central, yet unstudied, issue in evolutionary ecology is how animals adapt to changes in their nutritional environment to balance the costs and benefits of nutrient acquisition, allocation, and storage (1–3). This is of obvious relevance to modern humans but applies more generally where changes in climate, land use, and biodiversity alter an animal's nutritional environment. For instance, changes in atmospheric CO_2 concentration are likely to result in increased rates of photosynthesis and, hence, carbohydrate storage by plants (4). Insect herbivores are known to possess various short-term adaptive responses to the spatial and temporal variability that is typical of plants (5, 6), including compensatory feeding, selection of complementary foods, and changes in postingestive nutrient processing (1, 2). However, the question remains as to how populations adapt across generations in the face of persisting changes in patterns of nutrient availability and how the responses of different life stages are coordinated.

When faced with nutritionally imbalanced diets, compensatory feeding for the limiting nutrient results in overingestion of other nutrients, as is often seen when insects are confined to foods low in protein relative to carbohydrate (P/C) (1). Many animals, not just insects, regulate protein intake very tightly (7, 8) and so tend to ingest substantial excesses of energy when feeding on low P/C foods. Although insects are capable of dissipating a proportion of ingested energy excesses by increased thermogenesis (9, 10), sufficiently large surpluses may result in increased lipid storage and reduced fitness (2, 11). Whether fat storage is the direct cause of reduced fitness or a correlate of some other harmful effect of overeating carbohydrate has yet to be established for insects.

It might, therefore, be predicted that in persistently low-P/C environments, selection will operate to mitigate the costs of storing excess lipid. There are two traits that could change in response to selection: the amounts of such diets consumed and the way in which ingested nutrients are processed. Because the animal must consume adequate amounts of protein, and restricting intake to avoid ingesting excesses of carbohydrate will inevitably entail eating less protein, it follows that a likely outcome of selection in low-P/C environments would be phenotypes that dissipate excess carbohydrate rather than accumulate and store it as fat. In contrast, environments where there is a chronic shortage of carbohydrate should favor changes in metabolism that promote energy storage through lipogenesis. The balance between dissipation and storage has been shifted in genetically obese rodents (12), and the failure to dissipate, rather than store, energy in response to modern energy-rich diets has been implicated as an important causal factor in the human obesity epidemic (13).

Adapting to a changing nutritional environment may require coordination of phenotypic responses across different life stages. In the case of insect herbivores in which the larvae are the main feeding stages, not only must larval nutritional responses adapt, but the choice of egg-laying sites by adult females may also need to shift to track the evolving nutritional responses of the larvae, leading to a positive correlation between the preference of females to oviposit on a particular plant and the ability of the larvae to develop on that plant (14, 15). To date, evidence for such a positive relationship is contradictory, with many studies reporting only partial or negative correlations (16, 17).

Accordingly, we performed two separate, replicated laboratory quasi-natural selection experiments using the diamondback moth, *Plutella xylostella* L., to test the hypotheses that (*i*) the threshold for fat deposition in larvae is a target of selection against the costs of obesity and (*ii*) that female egg-laying preference will shift toward the diet to which the larvae have become adapted.

Results

Shift in Lipid Deposition on Artificial Diet Regimes. Larvae were reared for eight generations on either protein- or carbohydrate-biased artificial diets [mean composition by percent of dry mass of P/C of 45:7 and 14:38, respectively]. After one, four, and eight generations of selection, random cohorts of larvae were removed from each of the replicate lines, reared on a common "ancestral" diet of P/C of 26:26 for the first three larval stadia, and then placed for their final stadium on one of five test diets differing in P/C (P/Cs of 12:40, 19:33, 30:22, 40:12, and 47:5). Carbohy-
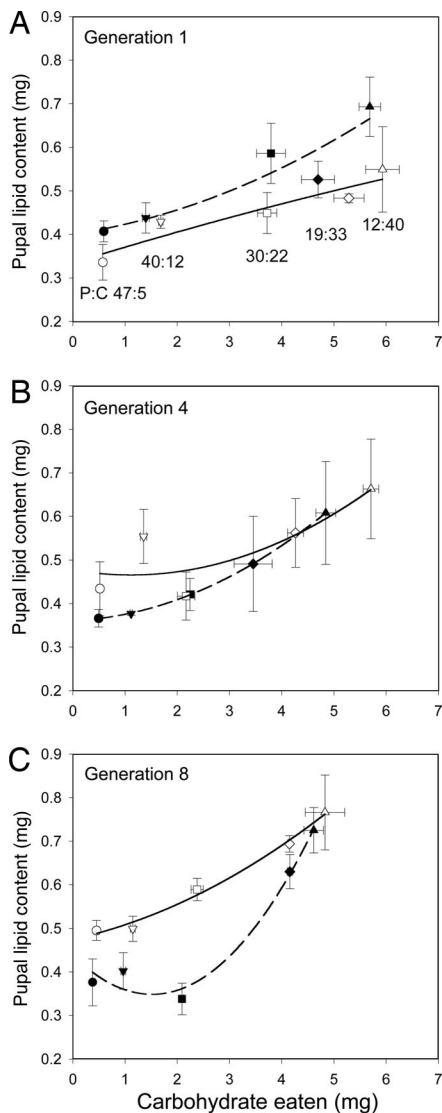
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Fig. 1. Carbohydrate utilization plots (18) for the artificial diet selection experiment. Points represent means ($\pm$SE) for each of the five test diets pooled across the three replicate lines within each selection regime ($n \approx 20$ for each data point). (*A–C*) Test diets are shown from left to right as follows, as P/Cs: 47:5, circles; 40:12, inverted triangles; 30:22, squares; 19:33, diamonds; and 12:40, triangles. Filled symbols and dashed lines correspond to the high-carbohydrate regime, whereas open symbols and solid lines correspond to the high-protein regime. Note that in some instances error bars are hidden by data points. Generations one (*A*), four (*B*), and eight (*C*) are shown. For each regime, quadratic regressions have been fitted to the data. Note how, as the experiment progressed, high-carbohydrate larvae ate more carbohydrate before they started laying down high levels of lipid, whereas high-protein larvae started laying down high levels of lipid at lower levels of carbohydrate consumption.

drate utilization was investigated by plotting pupal lipid content as a function of the amount of carbohydrate ingested throughout the final larval stadium.

As can be seen from Fig. 1*A*, larvae from both regimes responded to dietary carbohydrate in a very similar way after a single generation of selection. Body lipid content increased approximately linearly with increasing carbohydrate consumption. However, as the experiment progressed, and populations underwent further generations of selection, larval rearing history had an increasingly pronounced effect on carbohydrate utilization, with the two selection regimes diverging [$F_{2,11} =$

5.24, $P = 0.025$ for the interaction between dietary regime (carbohydrate- or protein-biased) and generation (one, four, or eight) in an analysis of covariance (ANCOVA), with dietary regime and generation as factors, mean pupal lipid content of each replicate line as dependent variable, and mean total carbohydrate consumption across the stadium per replicate line as covariate, having first established that there was no significant two- or three-way interaction between the covariate and the two factors ($P = 0.458$)]. This interaction term remained significant when differences in lean mass were controlled for by inclusion as a covariate ($F_{2,10} = 6.65, P = 0.015$). Larvae from the protein-biased regime (P/C of 45:7) responded by exhibiting higher levels of pupal lipid content at lower levels of ingested carbohydrate (Fig. 1 *B* and *C*). In contrast, high-carbohydrate-selected larvae progressively developed the ability to eat excess carbohydrate without laying it down as fat, as seen in the transition from a linear to a broken-stick relationship between carbohydrate consumption and pupal lipid content (Fig. 1 *A* vs. *C*). A change occurred over generations of selection in the total amount consumed of the test diets, as shown by a significant interaction between test diet and generation of selection ($F_{8,60} = 8.39, P < 0.0005$) when carbohydrate eaten was the dependent variable and regime, generation, and test diet were included as factors. In particular, there was a pronounced reduction from generation four to eight in the amount eaten of the P/C of 30:22 diet (Fig. 1 *B* vs. *C*). However, this pattern occurred in both selection regimes: hence, it apparently was unrelated to changes in lipid accumulation in response to dietary regime (interaction term regime by test diet: $F_{4,60} = 2.16$, $P = 0.085$; regime by generation by test diet: $F_{8,60} = 1.30, P = 0.261$).

Shift in Lipid Deposition on Nutritional Mutants of *Arabidopsis*. A second experiment mimicked the design of the first but used *Arabidopsis thaliana* starch-expression mutants in place of artificial diets. The use of plants expressing mutations in metabolic pathways provides an ideal framework for combining ecological realism with control over biochemical predictor variables (19). Insects were reared for eight generations on one of two *Arabidopsis* mutants, differing widely in their P/C profile. One of the mutants, phosphoglucomutase (*pgm*), is incapable of synthesizing starch (20). The other, *sex1*, is defective in its starch degradation and mobilization ability and hence contains an abundance of carbohydrate (21). In terms of macronutrient profile, *pgm* has a higher P/C than *sex1* (actual ratios were 1.5:1 and 0.4:1 for *pgm* and *sex1*, respectively).

After eight generations of selection, pupal lipid levels among the different selection regimes differed significantly (ANOVA: $F_{3,231} = 80.74, P < 0.0005$). Low-carbohydrate-selected (*pgm*) pupae stored significantly more lipid than high-starch-selected (*sex1*) pupae (Fig. 2). On average, low-starch-selected pupae contained $27.4 \pm 0.52\%$ lipid by mass, compared with $14.4 \pm 0.52\%$ (mean $\pm$ SE) for high-starch-selected pupae. This result is consistent with data from the artificial diet experiment, in which larvae on a high-carbohydrate regime evolved to convert less of their ingested carbohydrate to body fat. Lipid levels in pupae from a control line (unselected) were similar to those of the low-carbohydrate-selected pupae, and pupal lipid levels were similar within selection regimes, whether they had fed on the high- or low-starch mutant (ANOVA: $F_{1,231} = 0.10, P = 0.750$).

Shift in Oviposition Preference After Rearing on *Arabidopsis* Mutants. There was a significant effect of selection regime on host preference by ovipositing moths. Females from the low-starch-selection lines preferred to oviposit on their rearing host (two-way, one-sample *t* test: $t_{22} = 2.32, P = 0.030$), but females from the high-starch-selected and unselected lines showed no prefer-
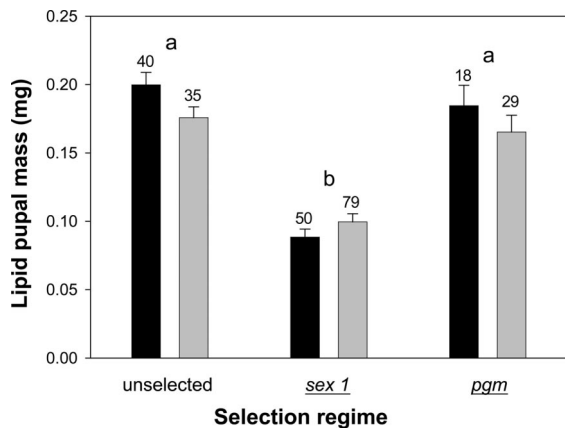
Fig. 2. Lipid content of pupae from the *Arabidopsis* mutant selection experiment. Larvae were taken from three regimes, unselected, selected on high-starch (*sex1*) *Arabidopsis* mutant, or selected on low-starch (*pgm*) *Arabidopsis* mutant and were tested on either of the two plant mutants (black bars represent the low-starch mutants, and gray bars represent the high-starch mutants). Lipid mass is presented as means ($\pm$SE), and numbers directly above the error bars indicate sample size. There was no difference in lipid content on the two mutants within a selection regime, but between regimes, significant differences were detected. Letters above bars refer to Tukey post hoc contrasts, and regimes with the same letter are not significantly different at $\alpha = 0.05$. Note how larvae reared for eight generations on the high-starch mutant (*sex1*) had substantially lower body lipid levels than the low-starch-reared (*pgm*) or unselected pupae.

ence for either mutant (two-way, one-sample *t* test: $t_{51} = -0.77$, $P = 0.446$; $t_{28} = 0.26$, $P = 0.797$, respectively) (Fig. 3).

Discussion

Eating excess carbohydrate relative to requirements leads to the deposition of high levels of body fat and reduced viability in larval insects (1), including Lepidoptera (2, 11), and the same is true of other animals, notably humans, in whom carbohydrate displaces fat oxidation, thus increasing fat storage. Our results indicate that restriction to a high-carbohydrate diet over multiple generations in *Plutella* leads to lowered deposition of body fat for the same consumption of carbohydrate, whereas restriction to a low-carbohydrate diet leads to enhanced fat storage. These results strongly imply that excess fat storage has a fitness cost and that such a cost is worth paying in environments where carbohydrate is scarce.

Two factors suggest a significant role for genetic adaptation in these responses, in addition to any part played by parental environmental effects (22). First, the *Arabidopsis* selection regimes made use of a common rearing environment for a complete generation before testing larvae on both hosts but nevertheless yielded results that corroborated those of the artificial diet experiment, where only the first three larval stadia before testing were on a common, ancestral diet. Such concordance makes an environmentally induced parental effect on offspring metabolism, akin to predictive adaptive responses in mammalian development (23, 24), an unlikely mechanism for explaining the patterns of fat accumulation observed during the artificial diet experiment. Second, the observed changes in lipid storage were directional and progressive; therefore, if parental effects were involved they must have been cumulative.

Given that larvae are the major feeding stage in the life history of *Plutella* and many other holometabolous insects, selection on larval nutrient use in response to diet will have an impact on the whole life history of the animal. Thus, when larval *Drosophila melanogaster* were artificially selected for low feeding rate, they deposited less fat before pupation and gave rise to adults that lived longer and had lower early-life fecundity than larvae selected for high rates of feeding (25). In reverse, when the focus of selection in *Drosophila* was adult longevity and early-life fecundity, larval metabolism changed in a similar manner, as if larval feeding rate had been selected (26). Similarly, selection for starvation resistance in adult *Drosophila* resulted in increased larval fat deposition but came at a cost of reduced larval survival (27).

Another key life history component is choice of egg-laying site by female moths. We found a shift toward a preference for laying eggs on the natal host in females reared for eight generations on the low-carbohydrate *pgm Arabidopsis* mutant, even after one generation of rearing on a different host (*Brassica napus*). In contrast, there was no significant shift toward preferential oviposition on the high-starch *sex1* mutant after selection on that plant. The observed response of oviposition behavior to selection, at least within the *pgm* population, indicates that females can distinguish between the two mutants. Whether moths were responding to levels of nutrients in (or on) the plants (28–30) or to correlated differences in plant secondary chemistry (31) is not known.

The nature of the mechanisms underlying the changes in carbohydrate and lipid metabolism in larval *Plutella* is as yet unknown but could involve an increase in respiration rate (9, 10), an increased ability to void excess carbohydrate from the gut, and/or a decrease in the efficiency with which digested carbohydrate is converted to stored lipids (32–34). Whatever the mechanisms, the demonstration that larval nutritional physiology and adult oviposition behavior respond rapidly to changes in the nutritional environment has implications not only for predicting responses of insect pest species to different plant cultivars and climate change (35), but also, more generally, for any heterotrophic organism that finds itself in an altered nutritional environment. Results suggest that, at least within the simplified setting of a laboratory environment, differences in plant macronutrient profiles may promote host-associated population divergence.

Materials and Methods

Artificial Diet Selection Experiment. An artificial diet strain of *P. xylostella* was obtained from the New York State Agricultural Experiment Station of Cornell University (Geneva, NY), under license. Two selection regimes were established, and each was replicated three times by using randomly selected founder populations of 200 adults. The high-protein regime presented larvae with a highly protein-biased foraging environment, whereas the high-carbohydrate regime was characterized by an abundance of dietary carbohydrate. Upon hatching, larvae were reared in 90-mm Petri dishes on a P/C of either 40:12 (high-protein lines) or 19:33 (high-carbohydrate lines) artificial diet (where P and C indicate the percentage by dry mass of protein and digestible carbohydrates in the diet) for their first two larval stadia. Artificial diets were based on an established laboratory recipe for *Plutella* (36). Upon reaching the third stadium, a random cohort of 150 larvae from each replicate selection line was transferred to a clear plastic feeding arena (173 × 115 × 60 mm) containing 100 diet blocks, each weighing ≈35 mg, spaced equidistantly across a 100 × 100-mm grid within the arena. The high-protein regime feeding arenas consisted of 75 highly protein-biased diet blocks (P/C of 47:5) and 25 protein-biased blocks (P/C of 40:12), whereas the high-carbohydrate regime consisted of 75 highly carbohydrate-biased blocks (P/C of 12:40) and 25 carbohydrate-biased blocks (P/C of 19:33). All diet blocks were replaced daily. Pupae were collected from arenas, and adults were mated within lines to found the next generation. All rearing was carried out in an incubator set at 26°C and 16 h of light/8 h of dark.

Selection regimes were continued for eight generations. After one, four, and eight generations of selection, a subset of eggs was removed from each replicate line, and resultant larvae were provided with the ancestral (control) diet of P/C of 26:26 until reaching the final stadium. Larvae were then presented with no-choice feeding bioassays, designed within the context of the geometric framework described in refs. 1 and 37. Freshly molted final-instar larvae were placed individually in 35-mm Petri dishes containing a disk of moistened filter paper and a preweighed block of diet. Five different test diets were used (P/Cs of 12:40, 19:33, 30:22, 40:12, and 47:5), and each was replicated eight times. Uneaten diet was removed from dishes daily, dried to constant mass, and weighed to 0.01 mg. Control diet blocks were also dried and weighed to provide regressions with which to calculate initial dry weights of diet blocks offered to larvae during feeding trials.

***Arabidopsis* Mutant Selection Experiment.** A laboratory *P. xylostella* colony was established by using wild-caught adults from Oxford and Chichester (U.K.). To allow adaptation to laboratory conditions, wild-caught moths were reared on seedling oil-seed rape (*B. napus*) in a controlled-temperature room at 26°C and 16 h of light/8 h of dark for 10 generations before starting selection experiments. Two selection regimes were used. Each of three replicate selection lines was founded with 100 pupae. In each case, ovipositing adults and feeding larvae were provided with either high-starch *A. thaliana* mutant plants (*sex1*) or low-starch mutants (*pgm*) for eight generations. Egg-bearing plants were transferred to $279 \times 159 \times 102$-mm plastic boxes fitted with fine-gauge steel mesh aeration panels, and larvae were provided with fresh plants ad libitum until pupation. After eight generations, larvae from each selection line were reared for an entire generation on a common, control diet of *B. napus*. This step was taken to help to ensure that any observed changes in larval performance could be attributed to genetic changes rather than to epigenetic phenomena such as maternal effects (22, 38, 39). Progeny of these *Brassica*-reared moths were then kept on *B. napus* until they reached the final larval stadium, at which point larvae were assigned to one of two treatments, either high- or low-starch mutants. Hence, larvae from both selection regimes were tested on both "host" and "novel" *Arabidopsis* mutants. Plants were replaced every 3 days until all surviving larvae had pupated. Pupal mass and lipid content were recorded for larvae reared on either test mutant and for unselected (*Brassica*-reared) controls.

Pupal Lipid Extraction. Pupae from both selection experiments were dried to constant mass at 40°C and weighed to within 0.01 mg. Dried pupae were placed individually into the wells of a Whatman (Brentford, Middlesex, U.K.) Multichem 96-well plate and covered with chloroform. After 8 h of soaking, the chloroform was removed with a suction pipette and replaced with fresh chloroform. This extraction process was repeated for a total of three washes. Pupae were then dried and weighed. Lipid content was calculated as prechloroform dry mass minus postchloroform dry mass.

Assessing Oviposition Preference in Lines Reared on *Arabidopsis* Mutants. Measures of oviposition preference were taken both at the start of the experiment (unselected population) and after eight generations of selection. As with the larval performance testing, selected populations were reared on *B. napus* seedlings for a single generation before oviposition testing. This was to ensure that any observed changes in oviposition preference could be attributed to genetic changes rather than to the Hopkins host-selection principle [preimaginal conditioning causes adults to preferentially oviposit on plants they experienced as larvae (40)] or chemical legacy [whereby chemical remnants of the larval-rearing environment bias oviposition preference toward the larval host (41)].

Oviposition preference was examined by two different choice tests using (*i*) excised leaves and (*ii*) whole plants. The experimental design for excised leaves involved removing two outer rosette leaves
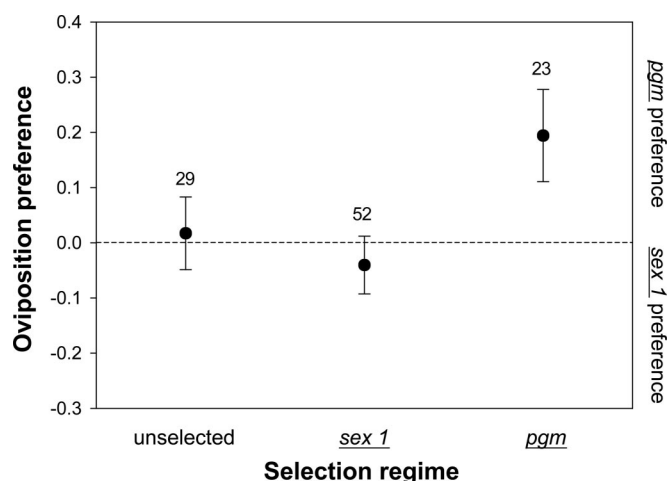

Fig. 3. Oviposition preferences in high-starch-selected (*sex1*), low-starch-selected (*pgm*), and unselected moths. Data indicate means (±SE) of Levene-adjusted oviposition scores. Positive values indicate a preference for the low-starch mutant, whereas negative numbers indicate a preference for the high-starch mutant. Numbers above error bars refer to the sample size of females tested.

from each of the *Arabidopsis* mutants, wrapping the leaf bases in cotton wool, and placing the base of each leaf into a 0.5-ml microcentrifuge tube (with the cap removed) filled with water. Four holes were then punched into the side of a 500-ml clear plastic tub, 5 cm above the base, such that they were equally spaced around the tub's circumference. The microcentrifuge tubes were inserted into the holes so that the rims of the tubes were flush with the inner edge of the tub and the leaves projected into the tub, adaxial surface uppermost. Leaves of each mutant, matched for size with those of the other mutant, were placed opposite each other. Clear lids were then added, and the tubs were placed under fluorescent lamps on the shelves in the constant temperature room. The four-leaf arrangement, combined with a 90° rotation of each tub with respect to the tub to its immediate left, ensured that positional influences upon oviposition preference were kept to a minimum (42, 43). *Plutella* pupae were sexed, and male/female pairs were kept separately in 5-ml glass vials. Vials were inspected daily, and each pair was added to a tub containing excised leaves the day after the pair was first observed in copula. Pairs were introduced into tubs at 1800 h and then removed at 0900 h the following morning, whereupon the number of eggs on each leaf was counted. Leaves were only used once.

For the choice test with whole plants, single plants of each mutant were matched for size and placed opposite each other in 500-ml plastic tubs. As with the excised leaf assays, precautions were taken to avoid positional effects. Pupae were paired, and mated adults were introduced into arenas as before. Eggs were counted on each plant. An initial analysis was conducted to determine whether the type of trial (excised leaves vs. whole plants) influenced oviposition preference. Trial type had no significant influence, whether as a main effect ($F_{1,96} = 1.22$, $P = 0.273$) or as an interaction with population ($F_{3,96} = 0.83$, $P = 0.482$). Hence, Levene-adjusted oviposition scores were pooled across trial types for all further analysis and graphical representation. The Levene statistic was calculated as $(x - y)/(x + y)$, where x is the total number of eggs laid on *pgm* plants and y is the number of eggs laid on *sex1*. Positive values of the statistic thus represent a preference for the *pgm* mutant. Individual populations of moths were analyzed by using two-way, one-sample *t* tests to determine whether they exhibited a significance preference for either mutant (Levene $\neq$ 0).

1. Raubenheimer D, Simpson SJ (1999) *Entomol Exp Appl* 91:67–82.
2. Simpson SJ, Sibly RM, Lee KP, Behmer ST, Raubenheimer D (2004) *Anim Behav* 68:1299–1311.
3. Stock MJ (1999) *Int J Obes* 23:1105–1117.
4. Harrington R, Woiwod IP, Sparks T (1999) *Trends Ecol Evol* 14:146–150.
5. Denno RF, McClure MS (1983) in *Variable Plants and Herbivores in Natural and Managed Systems*, eds Denno RF, McClure MS (Academic, New York), pp 1–12.
6. Whitham TG (1981) in *Insect Life History Patterns: Habitat and Geographic Variation*, eds Denno RF, Dingle H (Springer, New York), pp 9–27.
7. Simpson SJ, Batley R, Raubenheimer D (2003) *Appetite* 41:123–140.
8. Simpson SJ, Raubenheimer D (2005) *Obes Rev* 6:133–142.
9. Zanotto FP, Gouveia SM, Simpson SJ, Raubenheimer D, Calder PC (1997) *J Exp Biol* 200:2437–2448.
10. Trier TM, Mattson WJ (2003) *Environ Entomol* 32:1–8.
11. Raubenheimer D, Lee KP, Simpson SJ (2005) *Proc R Soc London Ser B* 272:2429–2434.
12. Johnson PR, Greenwood MRC, Horwitz BA, Stern JS (1991) *Annu Rev Nutr* 11:325–353.
13. Schoeller DA (2001) *Am J Clin Nutr* 73:511–516.
14. Thompson JN (1988) *Entomol Exp Appl* 47:3–14.
15. Courtney SP, Kibota TT (1990) in *Insect–Plant Interactions*, ed Bernays EA (CRC, Boca Raton, FL), Vol 2, pp 161–188.
16. Mayhew PJ (1997) *Oikos* 79:417–428.
17. Leather SR, Awmack CS (2002) *Oikos* 96:192–195.
18. Raubenheimer D, Simpson SJ (1994) *Funct Ecol* 8:783–791.
19. Adler LS, Karban R, Strauss SY (2001) *Ecology* 82:2032–2044.
20. Caspar T, Huber SC, Somerville C (1985) *Plant Physiol* 79:11–17.
21. Yu T-S, Kofler H, Häusler RE, Hille D, Flügge U-I, Zeeman SC, Smith AM, Kossmann J, Lloyd J, Ritte G, *et al.* (2001) *Plant Cell* 13:1907–1918.
22. Rotem K, Agrawal AA, Kott L (2003) *Ecol Entomol* 28:211–218.
23. Bateson P (2001) *Int J Epidemiol* 30:928–934.
24. Gluckman PD, Cutfield W, Hofman P, Hanson MA (2005) *Early Hum Dev* 81:51–59.
25. Foley PA, Luckinbill LS (2001) *Evolution (Lawrence, Kans)* 55:2493–2502.
26. Riha VF, Luckinbill LS (1996) *J Gerontol Ser A* 51:B284–B294.
27. Chippindale AK, Chu TJF, Rose MR (1996) *Evolution (Lawrence, Kans)* 50:753–766.
28. Städler E (1984) *Chemical Ecology of Insects*, eds Bell WJ, Carde RT (Sinauer, Sunderland, MA).
29. Ramaswamy SB (1994) in *Functional Dynamics of Phytophagous Insects*, ed Ananthakrishnan TN (Oxford & IBH, New Delhi), pp 55–78.
30. Derridj S, Wu BR, Stammitti L, Garrec JP, Derrien A (1996) *Entomol Exp Appl* 80:197–201.
31. Schulze W, Stitt M, Schulze ED, Neuhaus H, Fichtner K (1991) *Plant Physiol* 95:890–895.
32. Girard J, Ferre P, Foufelle F (1997) *Annu Rev Nutr* 17:325–352.
33. Downer RG (1985) in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, eds Kerkut GA, Gilbert LI (Pergamon, Oxford), pp 77–113.
34. Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Sanan DA, Raber J, Eckel RH, Farese RV (2000) *Nat Genet* 25:87–90.
35. Whittaker JB (2001) *J Ecol* 89:507–518.
36. Shelton AM, Cooley RJ, Kroening MK, Wilsey WT, Eigenbrode SD (1991) *J Entomol Sci* 26:17–26.
37. Simpson SJ, Raubenheimer D (1995) *J Insect Physiol* 41:545–553.
38. Rossiter M (1998) in *Genetic Structure and Local Adaptation in Natural Insect Populations*, eds Mopper S, Strauss SY (Chapman & Hall, New York), pp 113–138.
39. Mousseau TA, Fox CW (1998) *Trends Ecol Evol* 13:403–407.
40. Akhtar Y, Isman MB (2003) *J Chem Ecol* 29:1853–1870.
41. Corbett SA (1985) *Ecol Entomol* 10:143–153.
42. Singer MC (1986) in *Insect–Plant Interactions*, eds Miller JR, Miller TA (Springer, New York), pp 65–94.
43. Stanton ML (1979) *Oecologia* 39:79–91.

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