Integrative and Comparative Biology

Integrative and Comparative Biology, volume 53, number 2, pp. 329–339
doi:10.1093/icb/ict030

Society for Integrative and Comparative Biology

SYMPOSIUM

Plasticity in Learning Causes Immediate and Trans-Generational Changes in Allocation of Resources

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From the symposium "Ecological Epigenetics" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2013 at San Francisco, California.

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Synopsis Plasticity in the development and expression of behavior may allow organisms to cope with novel and rapidly changing environments. However, plasticity itself may depend on the developmental experiences of an individual. For instance, individuals reared in complex, enriched environments develop enhanced cognitive abilities as a result of increased synaptic connections and neurogenesis. This suggests that costs associated with behavioral plasticity—in particular, increased investment in "self" at the expense of reproduction—may also be flexible. Using butterflies as a system, this work tests whether allocation of resources changes as a result of experiences in "difficult" environments that require more investment in learning. We contrast allocation of resources among butterflies with experience in environments that vary in the need for learning. Butterflies with experience searching for novel (i.e., red) hosts, or searching in complex non-host environments, allocate more resources (protein and carbohydrate reserves) to their own flight muscle. In addition, butterflies with experience in these more difficult environments allocate more resources per individual offspring (i.e., egg size and/or lipid reserves). This results in a mother's experience having significant effects on the growth of her offspring (i.e., dry mass and wing length). A separate study showed this re-allocation of resources comes at the expense of lifetime fecundity. These results suggest that investment in learning, and associated changes in life history, can be adjusted depending on an individual's current need, and their offspring's future needs, for learning.

Introduction

Organisms today are confronted with rapid and pronounced environmental change, from the spread of invasive species and climatic change, to conversion of habitats to agriculture or cities (Palumbi 2001; Marzluff et al. 2008). Behavioral plasticity, the ability of a genotype to adjust the expression or development of behavior with respect to the environment, allows organisms to cope with such variable and novel environments (Sih et al. 2011; Tuomainen and Candolin 2011; Snell-Rood 2013). For instance, species with larger brains, a correlate of behavioral plasticity, are more likely to survive after introduction to a novel region or habitat, such as cities (Sol et al. 2005, 2008; Maklakov et al. 2011). Thus, biologists have long been interested in understanding the evolutionary forces that maintain variation in behavioral plasticity within and across species (Johnston 1982; DeWitt et al. 1998). Learning, the mechanism underlying developmental plasticity in behavior, has shown to be costly—species that invest more in learning and large brains breed later in life, suffer tradeoffs between learning, neural tissue, and other traits, and invest more in fewer offspring (Iwaniuk and Nelson 2003; Mery and Kawecki 2003; Isler and van Schaik 2006; Barrickman et al. 2008; Snell-Rood et al. 2011).

Although biologists often focus on variation in plasticity among genotypes, it is becoming increasingly clear that plasticity itself may be flexible depending on environmental conditions. In other words, the ability of a genotype to express a range of behavioral traits across environments may change with environmental conditions. In particular, a

Advanced Access publication April 26, 2013

plethora of laboratory studies indicate that rearing in environments enriched in social interactions and types of resources stimulates neural development and enhances adult learning ability (Renner and Rosenzweig 1987; Kolb and Whishaw 1998; van Praag et al. 2000; Olson et al. 2006), sometimes even into the next generation (Kiyono et al. 1985; Dell and Rose 1987). Thus, in complex environments in which learning may be more useful, organisms can facultatively alter investment in learning. Such effects of environmental enrichment have been documented across a range of systems, from insects to fish and mammals (Scotto Lomassese et al. 2000; Strand et al. 2010).

In predicting how plasticity will impact a population's response to a novel environment, biologists must consider not only genetic variation in plasticity (Ghalambor et al. 2007) but also environmentally induced variation in plasticity. The fact that learning itself varies with developmental conditions means that, in some cases, a plastic response to a novel environment may be even more pronounced than predicted on the basis of a population in its ancestral environment. Although it is clear that environmental enrichment stimulates behavioral plasticity, it is less clear whether it comes with the same life-history consequences as genetic variation in learning. The fact that learning itself is flexible is consistent with the idea that it is costly, otherwise organisms would invest in it maximally. However, we know little about the flexibility of such costs with respect to environmentally induced variation in learning.

This research focuses on the question of why learning ability itself is often flexible, by focusing on the hypothesis that increased investment comes with changes in life history toward a more "slow" k-selected life-history strategy. We use butterflies as a system because their learning is easy to measure (Traynier 1984; Papaj 1986; Hern et al. 1996; Smallegange et al. 2006) and there is significant variation in learning across families and environments (Snell-Rood and Papaj 2009). Female butterflies learn visual cues associated with finding locally abundant host plants (Papaj and Prokopy 1989; Hern et al. 1996). In addition, previous studies on the cabbage white butterfly, *Pieris rapae*, have identified conditions that require increased investment in learning for a given level of performance in finding hosts. First, females are innately biased toward searching for green colors, so they must invest more time in learning to search for novel-colored hosts, such as red hosts (Snell-Rood and Papaj 2009). Experience in a red host environment, relative to a green host environment, results in a greater change in performance over time (Snell-Rood and Papaj 2009), in addition to increases in the relative size of the mushroom bodies, a region of the insect brain important in learning (Snell-Rood et al. 2009). This suggests that experience searching for novel, red hosts results in greater investment in learning. Second, searching for hosts is more "difficult" in complex non-host environments, where a greater diversity or density of non-hosts is presented (Snell-Rood and Papaj 2009; Snell-Rood et al. 2009). Experience in a complex non-host environment relative to a simple non-host environment results in overall lower performance (Snell-Rood and Papaj 2009), in addition to increases in the relative size of the medulla, a region of the insect optic lobe (Snell-Rood et al. 2009). This suggests that experience searching in complex non-host environments is relatively challenging for a butterfly. In this work, we use *P. rapae* to study changes in resource allocation associated with experience searching in environments of varying difficulty (Fig. 1) where the less difficult environments are functionally treated as the control groups. Behavioral results of the different searching and learning experiences are presented in detail elsewhere (Snell-Rood and Papaj 2009; Snell-Rood et al. 2009, 2011), but summarized here. The present study investigates shifts to a "slower" life history by testing for increased investment in "self" at the expense of reproduction. Investment in self is measured as allocation of resources to the flight muscles (e.g., Boggs 1981), which trades off with reproduction in insects (Roff 1984; Zera and Denno 1997; Stjernholm et al. 2005). We additionally measured egg size, investment per egg, offspring size, and, in a separate study, lifetime fecundity (Snell-Rood et al. 2011). Our results show that flexibility in learning parallels flexibility in allocation of resources to self, which suggests that organisms adjust investment in costly learning based on the need for learning.

Methods

Learning experiences and experimental overview

Overview

Complete details of rearing and behavioral assays are reported elsewhere (Snell-Rood and Papaj 2009; Snell-Rood et al. 2009, 2011). Briefly, we collected gravid female *P. rapae* in the field and reared their offspring in a common garden design on artificial diet (modified methods of Troetschler et al. 1985; Webb and Shelton 1988). Mated adult female butterflies were allowed to search for hosts in one of four possible treatments, in which color of the host (green versus red) and non-host complexity (complex

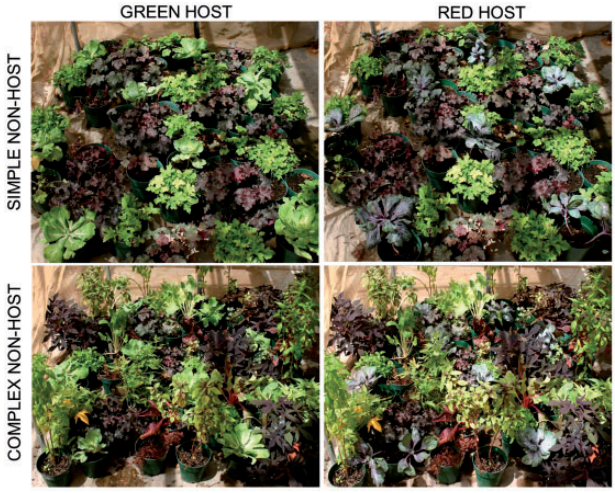

Fig. 1 Host-searching treatments. Female butterflies searched for either red or green hosts in either a simple or a complex non-host environment. Red hosts are rare in nature and more difficult for female butterflies to find because they have an innate bias to search for green colors. Previous studies have shown that female *P. rapae* searching in red host environments learn relatively more than those in green host environments, and also grow relatively larger mushroom bodies (regions of the brain involved in learning). Hosts are also more difficult to locate in complex non-host environments because there is a greater number and diversity of plants a female must learn to ignore when searching for hosts. Previous studies have shown that female *P. rapae* with experience searching in complex non-host environments grow larger optic lobes, relative to those with experience in simple non-host environments. This figure shows the setup for Experiment 2, in which the complex non-host treatment differed from the simple non-host treatment only in diversity (not density).

versus simple) were varied in a fully factorial manner (Fig. 1). Each full sibling group was measured in each environment, but individual females were only measured in one environment. Individual females were allowed to search for hosts for up to 2 h—females with at least 20 landings (on either hosts or non-hosts) were considered to have enough experience to use in measures of resource allocation. Previous analyses of these butterflies showed that females learn colors associated with host plants within 20 landings of experience in these host plant arrays (e.g., Snell-Rood and Papaj 2009), so we considered this an adequate minimum period of experience in a host plant array. After experience with finding hosts, butterflies were sacrificed for measures of resource allocation (Experiments 1 and 2) or maintained for 4 days for harvesting of eggs (Experiment 3). Because larval and adult butterflies had *ad lib* access to nutritionally complete diets, the results reported here are thought to be conservative, as resource tradeoffs should be less pronounced under such high resource conditions.

Experiment 1: effects of investment in learning on allocation of resources

Experiment 1 tested for effects of experience in searching for hosts on subsequent allocation of resources. Behavioral results of these trials were presented by Snell-Rood et al (2011). Briefly, this experiment used two hosts that differed in color and brightness, but not in leaf shape or nutritional quality to offspring (Slansky and Feeny 1977): green and red cabbage (two colors of *Brassica oleracea* var. *capitata*: Brassicaceae). The simple and complex non-host treatments varied in both the density and diversity of non-hosts. The simple treatment contained eight hosts of one color and 16 non-hosts, both red and green varieties of lettuce (*Lactuca sativa*: Asteraceae). The complex treatment contained eight hosts of one color and 40 non-hosts of 10 types: red and green varieties of lettuce (as in the simple treatment) in addition to red and green varieties of swiss chard (*Beta vulgaris* var. *cicla*: Amaranthaceae), basil (*Ocimum basilicum*: Lamiaceae), wood sorrel (*Oxalis stricta, O. rubra*: Oxalidaceae), and gaura (*Gaura lindheimeri*: Onagraceae). Between treatments, plants were spread over the same area. Eggs laid on host plants were removed from the plants in between trials. Between days of testing, all plants in the array were randomly shuffled in placement.

To measure changes in allocation of resources following learning, we sacrificed butterflies following their search for a host and measured their allocation of resources (details below) to ovaries or the thorax (at least four individuals per family). Only butterflies with at least 20 landings during their search were included in the present analyses. Controlling for variation in total number of landings during search for hosts did not change the results. Butterflies had *ad libitum* access to 15% honey water for 2–3 h following testing, prior to sacrifice.

Experiment 2: time-scale of changes in allocation of resources

Experiment 2 followed up the results of Experiment 1 by testing the time-scale of changes in allocation of resources following learning—were they a result of differences in use of resources during the test or changes in allocation of resources following the test? We ran butterflies through analogous behavioral tests, but sacrificed them either immediately following testing, or 5 h afterward. We focused on a 5-h time period because we assumed that it would be enough time for physiological changes in energy allocation to occur, given that a similar time period after host searching was sufficient to induce subtle

changes in brain morphology (Snell-Rood et al. 2009). The full details of these behavioral tests are presented by Snell-Rood and Papaj (2009). Briefly, butterflies searched for either red or green cabbage plants (as in Experiment 1), but the simple and complex environment varied only in diversity, not density, of non-hosts (Fig. 1). The simple environment contained red and green varieties of ragwort (Asteraceae: *Ligularia dentata*; total of 40 non-hosts and eight hosts), whereas the complex environment contained red and green varieties of five non-host species in equal numbers (total of 40 non-hosts and eight hosts; same species as in Experiment 1 except ragwort substituted for lettuce).

To determine a precise time-course of resource allocation under controlled situations, butterflies were either sacrificed immediately after testing, or 5 h following testing. In the latter treatment, butterflies were held without disturbance or food (to control for differences in acquisition of food) in a dimly-lit room for 5 h after testing; they were then sacrificed and held at −4°C until further analysis.

Experiment 3: Trans-generational effects of learning experiences on allocation of resources

We were interested in whether changes in allocation of resources to eggs, seen in Experiments 1 and 2, had effects on the development of the next generation. To test this idea, we harvested eggs from butterflies subjected to host-searching treatments in Experiment 1 (reported by Snell-Rood et al. 2011). Individual females experiencing at least 20 landings in a given host/non-host environment were housed for 3–4 days in a 2-l plastic cages with access to leaves of red and green cabbage (refreshed daily). Each female had *ad libitum* access to 15% honey water, presented in a plastic cup filled with a red or yellow scrub sponge. Cages were kept humid with a wet paper towel. Food was refreshed daily, and individual females were placed on their food each day to ensure they remained well-fed. Eggs were harvested daily and allowed to hatch on the host plant on which they were laid (held in individual cups in a climate chamber held at 22°C). Offspring were split between two artificial diets, which contained either dried red cabbage or dried green cabbage (20 g/l diet). Offspring were reared in groups of four on artificial diets in 4.5 oz plastic cups, at 22°C, 50% humidity, and 12:12 L:D. The majority of these offspring were sacrificed at emergence for measurements, although some were bred for future experiments.

Several measurements were taken on these offspring. Developmental time was measured as the number of days between deposition of the egg and emergence of the adult. Dry mass was measured on individuals sacrificed at emergence following 24 h of drying at 65°C. Wing-length was measured with calipers as the distance from the forewing articulation with the thorax to the apex of the wing. A different set of calipers that were less damaging were used on live butterflies to avoid injury, which resulted in slightly less precise measurements on the small subset of individuals measured live; in addition, there was slight variation in wing measurements between the two individuals making wing measurements. Thus, the statistical model investigating effects on wing-length controlled for both for the type of caliper, and the individual person performing the measurement.

Physiological measurements

Dissections of the thorax and ovary

For measurement of egg size and allocation of resources (Experiments 1 and 2), butterflies were stored in glassine envelopes encased in plastic bags at −4°C until analysis. Thoraxes were obtained by removing head, wings, and abdomen from each individual's thorax. Mature eggs were dissected out of females' abdomens, counted, and photographed (for measuring size) as in Snell-Rood et al. (2011). All oocytes and thoraxes were placed in individual vials, freeze-dried, and weighed prior to energetics analysis.

Quantification of lipid, protein, and carbohydrate

Amounts of lipids, free carbohydrates, glycogen, and protein were measured using modifications of existing methods developed by Van Handel (1965, 1985a, 1985b) and Van Handel and Day (1988), and subsequently modified in other studies (Olson et al. 2000; Telang and Wells 2004). Briefly, 200 μl of 2% sodium sulfate (Na_2SO_4) was added to each sample in a 1.7-ml microcentrifuge tube. Samples were pulverized with a pestle and vortexed. Five microliter aliquots of this solution were dispelled into 195 μl of 2% sodium sulfate solution in glass test tubes, and set aside for protein analyses. Eight hundred microliters of a 1:2 chloroform:methanol solution was added to the original sample, vortexed, and centrifuged for 20 min at 8000 *g*. Two 200-μl aliquots of the supernatant were dispelled into separate glass test tubes for analyses of free carbohydrates and lipids. Remaining supernatant was removed and the precipitate set aside for glycogen analyses. For each set of 30 samples, 10 standards (0–200 μl) were run; bovine serine albumin was used as a protein standard, trehalose as a free carbohydrate standard, pure glycogen

as the glycogen standard, and canola oil as the lipid standard. All standard tubes were brought to the same volume as the sample tubes.

To the protein samples, 1 ml of Bradford reagent was added; percent absorbance of the resulting mixture was measured in a spectrophotometer at 595 nm. To the tubes of carbohydrates, 2 ml of anthrone reagent (30 ml H_2O, 76 ml H_2SO_4, and 150 mg anthrone) was added. Samples from butterflies that had been allowed to freely forage (only Experiment 1) were allowed to react for 1 h at room temperature to measure fructose (Van Handel 1967), prior to continuation; subtracting fructose (gut sugars) from total free carbohydrates did not significantly change results. Test tubes were boiled for 10 min and then measured in the spectrophotometer at 625 nm. Lipid samples were allowed to dry under the hood for at least 3 h, prior to adding 200 μl concentrated sulfuric acid. These samples were then boiled for 2 min and 2 ml vanillin reagent was added (100 ml H_2O, 400 ml 85% H_3PO_4, and 0.6 g vanillin); samples were measured in a spectrophotometer at 525 nm. To the glycogen precipitate, 1.3 ml reduced-strength anthrone reagent was added (30 ml H_2O, 76 ml H_2SO_4, and 75 mg anthrone), prior to vortex and centrifugation for 10 min at 8000 *g*. One milliliter of this sample was transferred to a test tube and boiled for 10 min; samples were measured in a spectrophotometer at 625 nm.

Standard curves were fit to both raw and log-transformed measurements of standards. Log-transformed curves were a better fit for lipids, carbohydrates, and glycogen. Standard curves were calculated specific to each experiment and each protocol run; standard curves explained most of the variance in absorbance of standards ($R^2 > 0.93$; e.g., for Experiment 2: protein: amount: $F_1 = 1339.8$, $P < 0.0001$; date: $F_{11} = 2.19$, $P = 0.02$; full model: $N = 120$ samples, $R^2 = 0.93$, $F_{12,107} = 113.65$, $P < 0.0001$; carbohydrates: log amount: $F_1 = 1189.7$, $P < 0.0001$; date: $F_{11} = 4.85$, $P < 0.0001$; full model: $N = 108$ samples, $R^2 = 0.93$, $F_{12,95} = 103.95$, $P < 0.0001$; glycogen: log amount: $F_1 = 1296.9$, $P < 0.0001$; date: $F_{11} = 5.23$, $P < 0.0001$; full model: $N = 107$ samples, $R^2 = 0.94$, $F_{12,94} = 113.2$, $P < 0.0001$; lipids: log amount: $F_1 = 1489.5$, $P < 0.0001$; date: $F_{11} = 8.39$, $P < 0.0001$; full model: $N = 103$ samples, $R^2 = 0.95$, $F_{12,90} = 143.8$, $P < 0.0001$). Standard curves and aliquot sizes were used to calculate the amount of each nutrient in each sample, as standardized (divided) by the dry mass of each sample.

For assessing allocation of resources to the thorax, we focused on protein, free carbohydrates, and glycogen. For allocation to individual eggs, we focused on egg size, and the amount of lipid per egg (e.g., total lipids in a sample of eggs were adjusted for number and size of eggs).

Statistical analyses

ANOVAs in JMP 7.0 (SAS Institute, Cary, NC) were used to test for effects of individual experience in specific environments (host color, non-host complexity) on measures of resource allocation. Including hind-wing area in the models standardized for body size; this measure is correlated with other measurements of size such as length and mass of tarsi (Snell-Rood et al. 2009). In analyses of egg lipids, we controlled for total egg number and size by calculating the amount of lipid per unit egg size (cylindrical area) per egg.

Results

Experiment 1: allocation of resources in response to learning

Individuals with experience in searching for red hosts allocated more protein to their thorax and more lipids to their eggs several hours after their experience relative to butterflies with experience in searching for green hosts (Table 1 and Fig. 2), although the difference in allocation of lipids was only marginally significant. Individuals with experience in searching in the complex non-host environment—with a greater density and diversity of non-hosts—had significantly more carbohydrates and glycogen in their thorax and had larger eggs than did individuals with experience in the simple non-host environment (Table 1 and Fig. 2).

Experiment 2: time course of re-allocation of resources

The differences in allocation of resources observed in Experiment 1 could reflect differential use of resources during host search, or energy re-allocation following host search. To distinguish between these possibilities, in Experiment 2, butterflies were sacrificed either immediately following experience searching for hosts or 5 h following experience searching for hosts, during which time butterflies were kept in a cool, dark room without access to food, to control for differences in their intake of energy. There was a significant interaction between the color of the host and the time butterflies were sacrificed on the amount of lipids in the eggs of butterflies (Table 2 and Fig. 3): directly following learning, there was no difference between butterflies from either treatment in egg lipids, but 5 h later, butterflies that had learned to locate red hosts had significantly more

Table 1 Changes in allocation of resources following learning (Experiment 1)

	Host	NH	Family	Body size
Thorax protein	$F_{1,21} = 4.55$**	$F_{1,21} = 0.61$	$F_{6,21} = 1.98$	$F_{1,21} = 3.69$*
Thorax carbs	$F_{1,15} = 0.47$	$F_{1,15} = 7.93$***	$F_{6,15} = 2.10$	$F_{1,15} = 0.56$
Thorax glycogen	$F_{1,21} = 0.68$	$F_{1,21} = 6.01$**	$F_{6,21} = 1.71$	$F_{1,21} = 0.47$
Egg lipids	$F_{1,11} = 3.04$*	$F_{1,11} = 0.15$	$F_{5,11} = 0.88$	$F_{1,11} = 0.55$
Egg size	$F_{1,16} = 0.69$	$F_{1,16} = 5.50$**	$F_{5,16} = 2.25$*	$F_{1,16} = 0.83$

Female butterflies searched for either red or green hosts (Host) in a simple or complex non-host (NH) environment and were sacrificed 2–3 h following testing (during which time they had access to food). Shown are results from analyses of variance that also controlled for family (full sibling group) and body size (hind-wing area). Shown are *F* statistics from analyses of variance. *$P < 0.10$. **$P < 0.05$. ***$P < 0.01$.

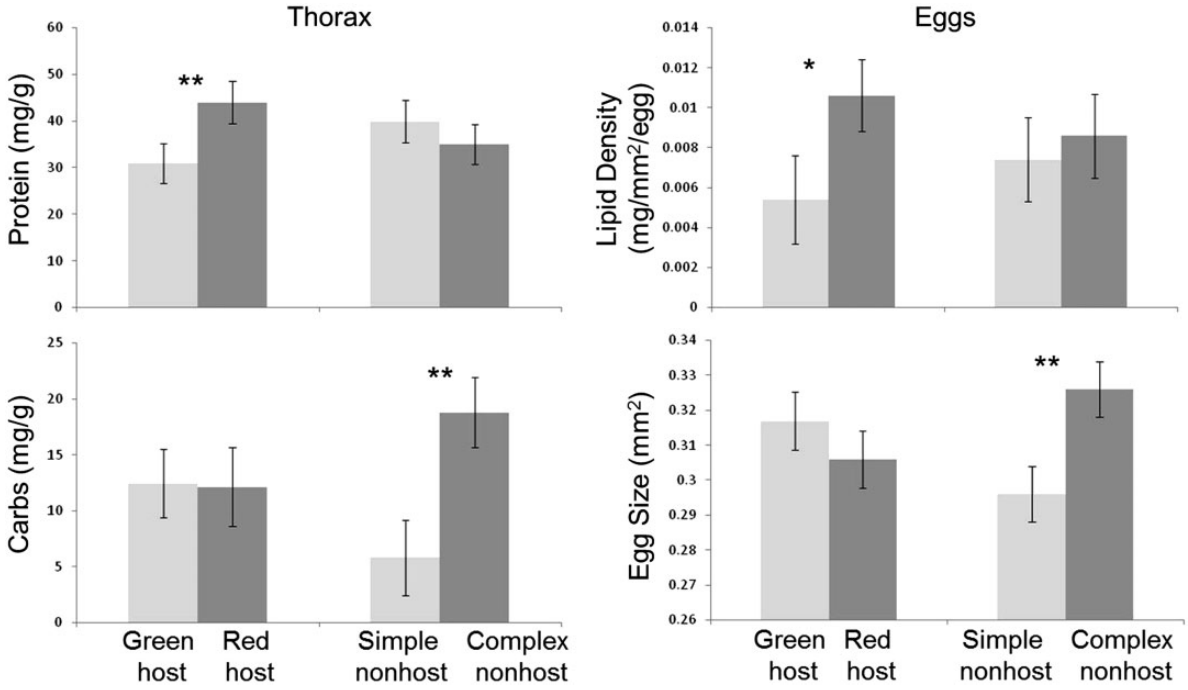

Fig. 2 Changes in allocation of resources following learning. Butterflies were sacrificed after experience in searching for either a red or a green host in either a simple or a complex environment (Experiment 1). Shown are least-square means (and standard errors) from analyses of variance controlling for body size and family (Table 1). Two asterisks represent $P < 0.05$; one asterisk represents marginal significance ($P < 0.10$).

lipids in their eggs (Fig. 3). There were similar patterns in thoracic protein levels, although here the interaction was only marginally significant (Table 2 and Fig. 3). The effects of non-host environment observed in Experiment 1 were not observed in Experiment 2, likely because simple and complex environments in Experiment 1 differed both in density and diversity of non-hosts, while in Experiment 2, they differed only in diversity of non-hosts.

Experiment 3: trans-generational effects of re-allocation of resources

To determine whether re-allocation of resources following experience with hosts affected the fitness of offspring, eggs of females were harvested on red and green cabbage following their host-searching experience (Experiment 1) and larvae were reared on artificial diets containing either red or green cabbage flour (in a two by two design for each mother; see "Methods" section). The body size (wing length and dry mass) and the developmental time of offspring were strongly influenced by the identity of their mother and maternal grandmother. However, the original host-searching experience of an offspring's mother also had a significant effect on offspring development. Mothers given experience in a red host environment (relative to a green host environment) produced offspring with a larger

Table 2 Re-allocation of resources following experience in searching for hosts (Experiment 2)

	Host color	NH complexity	Treatment	TM × host	Body size
Thorax protein	$F_{1,15} = 2.92$*	$F_{1,15} = 0.12$	$F_{1,15} = 1.25$	$F_{1,15} = 2.94$*	$F_{1,15} = 2.48$
Thorax carbs	$F_{1,15} = 0.00$	$F_{1,15} = 0.91$	$F_{1,15} = 1.81$	$F_{1,15} = 0.03$	$F_{1,15} = 0.27$
Thorax glycogen	$F_{1,15} = 0.05$	$F_{1,15} = 0.45$	$F_{1,15} = 1.64$	$F_{1,15} = 2.45$	$F_{1,15} = 2.33$
Egg lipids	$F_{1,22} = 2.31$	$F_{1,22} = 0.04$	$F_{1,22} = 2.58$	$F_{1,22} = 6.39$**	$F_{1,22} = 0.21$
Egg size	$F_{1,23} = 0.29$	$F_{1,23} = 0.03$	$F_{1,23} = 0.39$	$F_{1,23} = 0.86$	$F_{1,23} = 0.13$

Female butterflies learned to locate either red or green hosts (host color) in a simple or complex non-host (NH complexity) environment. Butterflies were sacrificed either directly after learning, or 5 h following learning after rest without food (treatment). Shown are *F* statistics from analyses of variance that also controlled for body size (hindwing area). We were particularly interested in an interaction between the difficulty in searching (e.g., red versus green host) and the time since the experience concluded (Tm × host). There were no significant interactions between treatment and non-host complexity. *$P < 0.10$. **$P < 0.05$.

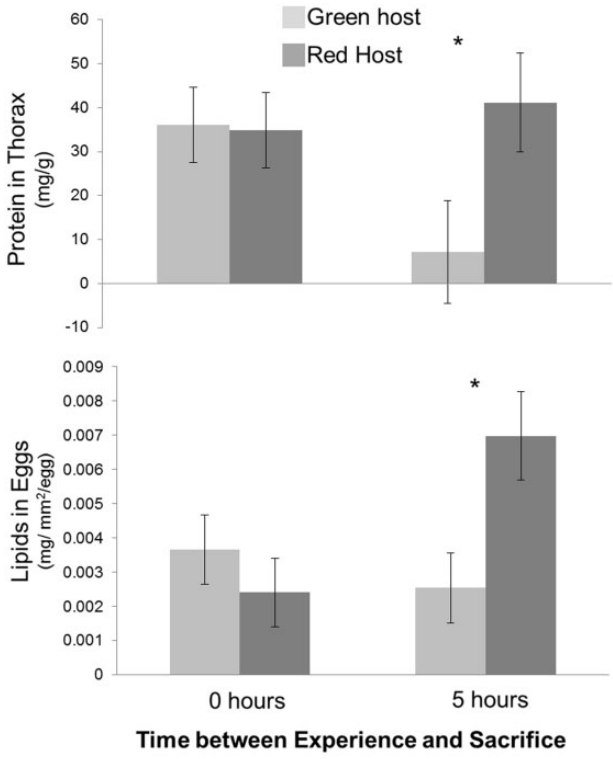

Fig. 3 Re-allocation of resources following learning. Butterflies were sacrificed either immediately following their search for hosts, or 5 h afterward, during which time they were resting without food (Experiment 2). Shown are least-square means (and standard errors) from analyses of variance controlling for body size and family. Near-zero values result from the fact that standard curves are less accurate close to zero (Table 2). One asterisk represents $P < 0.05$.

adult dry mass (Table 3 and Fig. 4). Mothers with experience in a complex non-host environment (relative to a simple non-host environment) produced offspring with longer wing length (Table 3 and Fig. 4).

Discussion

Learning experiences lead to adaptive re-allocation of resources

We found that allocation of resources was remarkably flexible, and responded to the prior experience of an individual, with long-term consequences across generations. In particular, if an individual had to invest more in learning, relatively more resources were invested in individual development, as seen in increased resource allocation to the thorax and to each individual egg. Cabbage white butterflies with experience with red hosts or with complex non-host environments allocated significantly more resources to the thorax (protein or carbohydrates) and invested more resources per offspring (lipid density and/or egg size; Table 1 and Fig. 2) relative to those that learned to search for green hosts or in a simple non-host environment. Offspring of these butterflies were larger than were those of butterflies that learned to locate green hosts or learned within a simple non-host environment (Table 3 and Fig. 4). We also found that differences in resource allocation following learning were not due to differential depletion of resources but instead due to changes in allocation of resources in the hours following learning (Table 2 and Fig. 3). In particular, it appears that experience with green hosts results in a decrease in thorax protein investment, whereas experience with red hosts results in an increase in egg investment (Fig. 3). It is important to note that increased investment in "self" and learning can be a function of either increased investment in the learning process itself (e.g., the energetic costs associated with forming memories or having a large brain) or increased investment in the searching and information gathering important for the learning process (e.g., the resources associated with more flight).

Table 3 Offspring development is affected by the searching experience of their mother (Experiment 3)

	Dam[a]	Granddam	Sex	Diet 1	Diet 2	Host	NH
Dvpmt	$F_{26,2237} = 19.3$***	$F_{11,2237} = 17.6$***	$F_{1,2237} = 0.69$	$F_{1,2237} = 9.0$**	$F_{1,2237} = 0.03$	$F_{1,2237} = 0.52$	$F_{1,2237} = 0.00$
Mass	$F_{26,1798} = 10.6$***	$F_{10,1798} = 13.8$***	$F_{1,1798} = 34.0$***	$F_{1,1798} = 0.02$	$F_{1,1798} = 17.2$***	$F_{1,1798} = 9.17$*	$F_{1,1798} = 3.31$
Wing	$F_{26,2225} = 30.3$***	$F_{11,2225} = 57.3$***	$F_{1,2225} = 946$***	$F_{1,2225} = 4.32$*	$F_{1,2225} = 4.28$*	$F_{1,2225} = 0.01$	$F_{1,2225} = 17.5$***

"Dam" refers to female parent of an individual, whereas granddam is the individual's grandmother (i.e., family in Table 1). Female parents were subjected to various learning environments ("Host" = red or green cabbage; "NH" = simple or complex non-host environment) prior to collection of eggs both on green and red cabbage (Diet 1). First instar larvae were transferred from their first diet (the host on which the eggs from which they hatched were deposited) to either a green or red cabbage artificial diet (Diet 2). Several measures were taken on offspring, including their developmental time (from oviposition to date of emergence from the pupa) wing length, and dry mass (following 48 h in a drying oven). Shown are *F* statistics from analyses of variance that also controlled for sex. [a]Nested within "Granddam, host color, and non-host complexity". *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

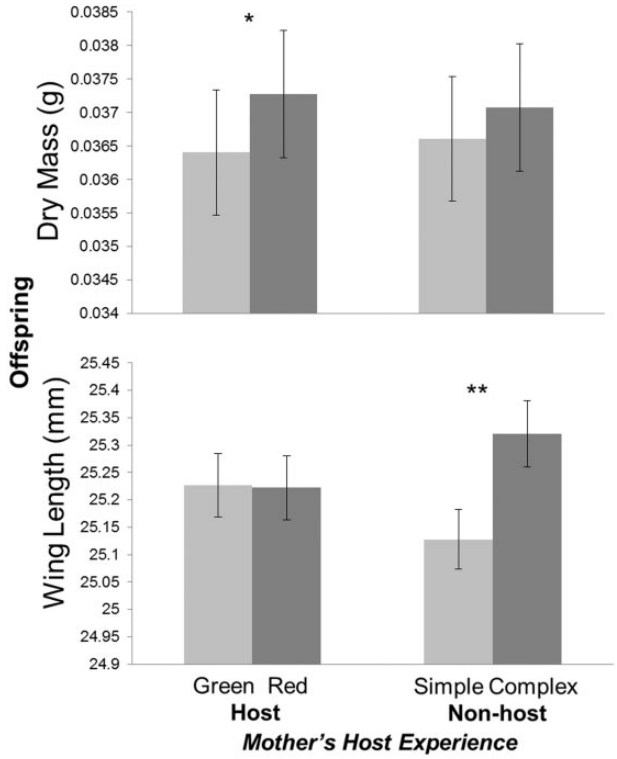

Fig. 4 Maternal experience in searching for hosts affects offspring development. Females learned to locate either red or green hosts in simple or complex non-host environments (Experiment 1). Their offspring were reared (Experiment 3) on diet containing extract from either red or green cabbage (half on each). Adult body size measurements were taken on offspring. Shown are least-square means (and standard errors) from analyses of variance that also controlled for dam, granddam, sex, the host on which the egg was laid, and the diet of the larvae (Table 3). One asterisk represents $P < 0.05$, two represent $P < 0.01$.

At first, these results suggest that learning may not be as costly as previous studies have indicated. However, although *P. rapae* are investing more in themselves and in individual offspring, an additional dataset from these butterflies showed that such allocation of resources comes at the expense of total fecundity. Individual butterflies with experience in looking for red hosts or searching in complex non-host environments have significantly lower lifetime fecundity—on the order of 30% fewer eggs (Snell-Rood et al. 2011). These tradeoffs between reproductive investment and thoracic resources recall other studies in insects showing tradeoffs between reproduction and flight muscles (Roff 1984; Zera and Denno 1997), reproduction and energy reserves (Brough and Dixon 1989; Djawdan et al. 1996; Wheeler 1996), or egg number and egg size (Fox and Czesak 2000).

Why might increased investment in learning favor increased investment of protein and carbohydrates in flight muscles? Free carbohydrates (e.g., trehalose) and storage carbohydrates (e.g., glycogen) are major short-distance fuels for flight in butterflies (Wyatt 1967; Sidhu et al. 1984; Rankin and Burchsted 1992; Thompson 2003) and may fuel the larger amount of flight during learning and during searches for hosts. Increased investment in flight muscle (protein) or fuel for flight (carbohydrates) may aid this searching and learning, or additional searching for hosts later in adulthood. An alternate explanation is that butterflies in more "difficult" environments increased thoracic investment, thereby being able to disperse more effectively from a less preferred area (Merckx and Van Dyck 2006; Pellegroms et al. 2009). However, the fact that butterflies that invest in learning in difficult environments (e.g., red hosts) end up finding as many host plants as those in "easier" (e.g., green host) environments (Snell-Rood and Papaj 2009) suggests that individuals do not have to leave in order to find a large number of hosts. In addition, the hosts used in these experiments are nutritionally comparable (Slansky and Feeny 1977), so a female's fitness would benefit from staying, at least in the red-host environment.

Experience in red host environments and complex non-hosts environments induced somewhat different changes in allocation of resources. It is not clear why non-host complexity would primarily affect carbohydrates (Table 1 and Fig. 2), while host color would primarily affect protein (Tables 1 and 2; Figs. 2 and 3), although the increased search costs associated with complex environments (Fig. 1) might favor increased energy available for flight.

Our results also suggest that cabbage white butterflies with more experience in difficult environments invested more in individual offspring, which had effects on the next generation. Why might increased egg size and lipid content improve performance of offspring? Increased lipid reserves are essential for longevity and resistance to starvation (Chippindale et al. 1996; Ellers 1996; Zera and Harshman 2001). If females perceive a novel, unpredictable, or otherwise "difficult" environment, increasing investment in each offspring may improve the likelihood those offspring survive similar conditions. In this experiment, where conditions for rearing larvae were optimal (i.e., *ad lib* artificial diet), this may have translated into larger body size of adults (Fig. 4).

Together, these results suggest that plasticity—both in behavior and physiology—may facilitate the use of rare or novel environments, in this case, host plants. This proposition recalls observations on other insects. For instance, in seed beetles, individuals with larger eggs (induced by exposure to a particular native host) are better able to utilize a novel, non-native host plant (Fox and Savalli 2000), and in *P. rapae*, mothers adaptively change allocation per offspring depending on their larval experience (e.g., on a poor quality host) (Rotem et al. 2003). Such behavioral and physiological plasticity may explain why so many butterflies have been able to shift to different host plants and to incorporate a range of novel, non-native hosts (Graves and Shapiro 2003).

Implications and future directions

Overall, the results of this work suggest that allocation of resources to learning or reproduction is remarkably flexible. Such flexibility in the costs and benefits of plasticity increases the chances that learning may evolve. In this case, flexibility in ovarian development, which is common in insects (Papaj 2000), may allow the benefits of learning to be realized in rare or complex environments. This work bolsters the idea that innate biases to use commonly encountered environments may significantly expand the conditions under which learning may evolve (Getty 1996; Snell-Rood and Papaj 2009) because the costs of learning are only experienced in rarely encountered environments where the benefits of learning are realized.

Studies on flexibility in learning suggest that "hidden" reaction norms that are revealed in novel environments (Ghalambor et al. 2007) may tend to be steep and adaptive. In predicting how species will respond to novel and changing environments, it is possible that in some cases, realized plasticity may be greater than that suggested by current or ancestral populations. Likewise, the costs of plasticity—increased investment in self at the expense of reproduction—may be more pronounced in these environments. Given that the rate of population growth, and thus fecundity, is related to evolutionary potential of populations in novel environments (Lande 1998; Reznick and Ghalambor 2001), re-allocation of resources into plasticity at the expense of reproduction would potentially impede the evolutionary response of a population in novel environments, but that is an area ripe for future research.

The effects of enriched and novel environments on a wide range of phenotypes, from resource allocation (Figs. 2–4), to neural development (Snell-Rood et al. 2009), to reproduction (Snell-Rood et al. 2011), suggest that hormonal processes may be involved in the observed differences between environments. Indeed, it is possible that generalized responses to stress may mediate adaptive responses of learning and allocation of resources in novel environments—increased investment in learning via diversion of resources away from reproduction may allow individuals to cope with unexpected, extreme, or novel conditions (McEwen and Wingfield 2003). The long-term impacts of enriched and novel environments suggest that epigenetic processes may also be involved, either through potentially heritable alterations of gene expression, or through maternal effects such as changes in resources deposited in eggs. Indeed, empirical evidence in vertebrates suggests that DNA methylation and histone acetylation may be involved in changes in neural development in response to environmental enrichment (Borrelli et al. 2008; Miller et al. 2008; Fagiolini et al. 2009; Kuzumaki et al. 2011), even across generations (Arai and Feig 2011). Understanding the role of stress and epigenetics in developmental responses to novel environments is an exciting area of current and future research (e.g., Badyaev 2005).

Acknowledgments

Many thanks to Bonnie Bell and Noel Eloriaga for help with dissections of ovaries, protein, and lipid

analyses, and measurements of body size and egg size. Much appreciation is owed to Art Shapiro, Adam Porter, and Nathan Morehouse for contributing butterflies and to Phil Foster, Sue, and Dan Oliver for help with collecting. Abreeza Zegeer, Bill Birky, Andrew Stoehr, Katie Massie, Demetre Sirakis, Natasha Pearce, and Jacob Schmidt helped with the logistics and labor of butterfly care.

Funding

This work was supported by the Center for Insect Science (University of Arizona), the Animal Behavior Society, Sigma Xi, the Department of Ecology and Evolutionary Biology at the University of Arizona, and the Philanthropic Educational Organization. E.S.R. was funded on an NSF graduate research fellowship during the initial design of this work.

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