

Comparative elemental stoichiometry of ecologically diverse *Drosophila*

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Summary

1. Chemical analyses of ecologically diverse species of *Drosophila* reveal substantial variation among them in their nitrogen and phosphorus contents, determined as a percentage of adult dry mass. Across *Drosophila* species, the nitrogen content of flies is positively correlated with phosphorus content.
2. For both N and P, the concentration in the flies is correlated with that of their breeding sites. The variation in the elemental composition of breeding sites, and therefore of the flies themselves, may affect the ecology and life-history variation of *Drosophila*.
3. The range of variation in N and P contents and the N : P ratio is considerably less among *Drosophila* than among their breeding sites, indicating that there is substantial constancy for elemental composition across *Drosophila* species. The N : P ratio varies somewhat among species, indicating that the nitrogen and phosphorus contents do not change in perfect proportion across species.
4. An important, but unanswered, question concerns the relation between intraspecific homeostasis and interspecific constancy for elemental composition. Three possible mechanisms by which such interspecific constancy is brought about are discussed.

Key-words: Consumers, homeostasis, nitrogen, phosphorus, resources

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Introduction

Because organisms and the resources they consume often differ dramatically in elemental composition, trophic interactions can entail major adjustments in both the absolute and relative abundance of different elements. For example, because the carbon to nitrogen ratio of herbivorous insects is much higher than that of the plants they feed on, resource processing by these consumers entails concentration of nitrogen from the food and excretion of excess carbon (Scriber & Slansky 1981). Such differences in elemental composition between resources and consumers are the basis for the emerging field of ecological stoichiometry (Sterner & Elser 2002).

In general, the stoichiometric challenge to an organism may depend on (1) the difference in elemental composition between the organism and its resources; (2) the range of variation in the composition of its resources; and (3) the degree to which its own body composition is constrained to lie within specific limits. For example, a parasitoid wasp that specializes on a single species of insect host probably experiences little stoichiometric challenge, because the wasp and its host are likely to be very similar in body composition and

because there is little variation within the host population in body composition. Thus, the wasp predictably has access to resources that are very similar in body composition to itself. In contrast, the caterpillars of a polyphagous species of butterfly face much greater stoichiometric challenges, because their body composition is very different from that of their host plants and because the different species of host plants are likely to vary in elemental composition.

There is likely to be an interaction between the second and third factors in the challenge they pose to an organism, as individuals within a stoichiometrically flexible species feeding on variable resources may often end up with a suboptimal body composition. Thus, there may be a stoichiometric trade-off: should an organism have a flexible body composition, entailing relatively flexible allocation of resources but reduced physiological functioning, or should it have a more fixed body composition, which would allow more optimal functioning but more stringent resource processing? For instance, species-specific stoichiometric constraints in some phytophagous insects and freshwater zooplankton lead to a positive correlation between the growth rates or growth efficiencies of these consumers and the nutrient (N or P) content of their resources (Scriber & Slansky 1981; Elser *et al.* 2000a). Given the broad potential significance of such stoichiometric

effects, it is important to examine how variation in the nutrient content of resources affects the biology of other groups of organisms.

In the present study, we consider consumers that differ greatly in elemental composition from their resources, which in turn exhibit substantial variation in nutrient content. Given that these consumers are faced with challenges 1 and 2 above, how have they resolved issue 3, i.e. how flexible are they in body content? Because stoichiometric limitations on organismal function are likely to vary among different groups of organisms, we focus on species belonging to a single ecologically diverse genus, *Drosophila*. Among *Drosophila* species, an initial survey of frugivorous and cactophilic species showed that the nitrogen and phosphorus content of adult flies differed considerably, with the frugivorous species being relatively enriched in both elements (Markow *et al.* 1999). Intriguingly, the nutrient content of the larval food resources and the flies differed in the same direction, i.e. fruits were found to be richer in both nitrogen and phosphorus than cactus tissue.

In order to determine whether or not this covariance can be observed more generally across *Drosophila* species requires assessment of additional species whose breeding sites span a greater range of nutrient contents than those considered in Markow *et al.* (1999). The species considered in the present analysis are ecologically and phylogenetically representative of the diversity within the genus *Drosophila*. Our sample includes species that breed in fruits, mushrooms, decaying vegetation, cactus necroses and sap exudates; and it includes members belonging to six different species groups within the two main subgenera, *Sophophora* and *Drosophila*, which are thought to have diverged ~61–65 Mya (Powell 1997). Our results reinforce the conclusion that the body composition of flies is correlated with that of their larval food resources. However, our results also show that the elemental composition of *Drosophila* is evolutionarily constrained to lie within much narrower limits than that of their resources. Such constraints, if found to be widespread, may be of considerable importance in the ecology and evolution of these species.

Materials and methods

SAMPLES

Owing to the logistical challenges of collecting *Drosophila* and their resources in the wild, collection methods varied somewhat among the different species examined, but in all cases both the flies and their known breeding sites were analysed. *Drosophila quinaria* Loew were reared from skunk cabbage (*Symplocarpus foetidus* (L.) Nutt.) collected from natural populations in Monroe County, New York in June 1998. Skunk cabbages are the principal breeding site of *D. quinaria* (Jaenike 1978).

Adults of *Drosophila carbonaria* Patterson and Wheeler were aspirated directly from their mesquite

(*Prosopis glandulosa* Torr.) host in southern Arizona in March 2001. These flies feed exclusively on the fluxes (sap exudates) of mesquite, which were collected in Eppendorf tubes as they dripped from trees.

Two species of mycophagous *Drosophila* were examined. *Drosophila neotestacea* Grimaldi, James and Jaenike were collected by sweep netting adult flies that had been attracted to *Agaricus bisporus* (Lange) Imbach mushrooms that had been set out as baits in Herkimer County, New York in August 1998. The mushrooms were then collected from the field and brought to the laboratory and emerging flies collected. *Drosophila recens* Wheeler were reared in the laboratory at 22 °C on *Agaricus bisporus*.

ELEMENTAL ANALYSES

For all species, flies were dried at 60 °C for 72 h and resource samples from their breeding sites were dried at 60 °C for 1 week, after which they were ground to a fine powder for assaying N and P content. Although male and female flies were analysed separately, in all cases their elemental compositions were very similar. Each *Drosophila* sample consisted of 30–50 flies pooled. Therefore, we present the elemental composition of the *Drosophila* species averaged across the two sexes. In all cases, results are reported in units of mg N or mg P per mg dry mass.

Total nitrogen contents of flies and their breeding sites were determined using a Europa Scientific 20/20 mass spectrometer (Cheshire, UK) using air as the standard (Markow *et al.* 1999). Total phosphorus was determined using persulphate oxidation followed by the analysis of orthophosphate using the acid molybdate technique (APHA 1992; Markow *et al.* 1999). As previous analyses have yielded virtually identical results among replicate runs of the same material (Markow *et al.* 1999), in the present study each sample was run only once.

The N and P contents of the different *Drosophila* species were examined as a function of the N and P contents of their breeding sites. *Drosophila* species differ not only in their breeding sites, but also in body size and therefore their surface area to mass ratios. This may cause variation among species in the fraction of their body mass made up of cuticle, one component of which is chitin, which is ~7% nitrogen by mass. Consequently, if the body sizes of *Drosophila* are not independent of the types of breeding sites they utilize, this could lead to body size-mediated correlations between the N and P contents of flies and their breeding sites. Therefore, both breeding site nutrient composition and *Drosophila* body size were entered as independent variables in an analysis of the flies' N and P contents.

Results

The N and P contents of the mycophagous species *D. neotestacea* and *D. recens*, the skunk cabbage specialist

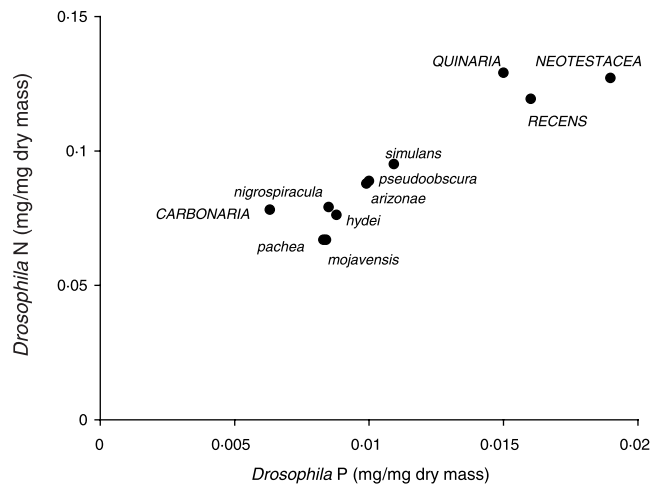


Fig. 1. Correlation between N and P content of 11 species of *Drosophila*, as a percentage of the dry mass of the flies (sexes pooled). Data from Markow *et al.* (1999) identified by lower case labels.

D. quinaria and the mesquite-flux specialist *D. carbonaria* are presented in Fig. 1, along with the N and P contents of other *Drosophila* species obtained in earlier studies of cactophilic and frugivorous species (Markow *et al.* 1999). The data reveal two-fold variation among *Drosophila* species in N content (as a fraction of dry mass) and three-fold variation in P content. Furthermore, the N and P contents of the flies are highly correlated across *Drosophila* species ($r = 0.93$, 11 species, $P < 0.001$). Thus, flies that are enriched in one element tend to be enriched in the other.

The N and P contents of the *Drosophila* species are also correlated with the N and P contents of their breeding sites (Fig. 2). In mixed stepwise regression models (JMP version 4.0.4, SAS Institute 2001), the N and P contents of the 11 species of *Drosophila* considered here were significantly correlated with those

of their breeding sites (nitrogen: $r^2 = 0.56$, $F = 11.3$, $df = 9$, $P = 0.008$; phosphorus: $r^2 = 0.77$, $F = 30.9$, $df = 9$, $P = 0.0004$), but there was no effect of fly body size, as measured by thorax length, on fly nutrient content (nitrogen: $r^2 = 0.005$, $F = 0.9$, $df = 9$, $P = 0.38$; phosphorus: $r^2 = 0.03$, $F = 1.0$, $df = 9$, $P = 0.35$). Thus, the correlation between the nutrient content of flies and that of their breeding sites is not due to difference in the body sizes of flies using different types of resources.

Although the N and P contents of the *Drosophila* species are positively correlated with those of their breeding sites, the flies exhibit much less stoichiometric variation than do their resources. Sterner & Elser (2002) propose a method by which the degree of intraspecific homeostasis in elemental composition can be quantified by plotting on a logarithmic scale the nutrient composition of consumers as a function of the nutrient composition of their resources. The degree of homeostasis, H , is defined as the inverse of the slope of such a regression. Thus, H ranges from 1, where the nutrient content of consumers is directly proportional to that of their resources, to ∞ where the body composition of consumers is independent of their food resources. Values of $H > 1$ indicate that the body composition of the consumers is homeostatically regulated.

Here we plot, on a logarithmic scale, the nutrient content of different *Drosophila* species as a function of the nutrient content of their resources. The inverse of the slope of the regression is a measure of 'interspecific constancy' in elemental composition, symbolized by the parameter C . The data reveal considerable interspecific constancy in elemental composition, as the flies are much more similar to each other than are their resources (Fig. 3). The slopes (SE) of these regressions are 0.163 ± 0.065 and 0.162 ± 0.047 for N and P, respectively, yielding values of $C = 6.14$ for N and 6.17

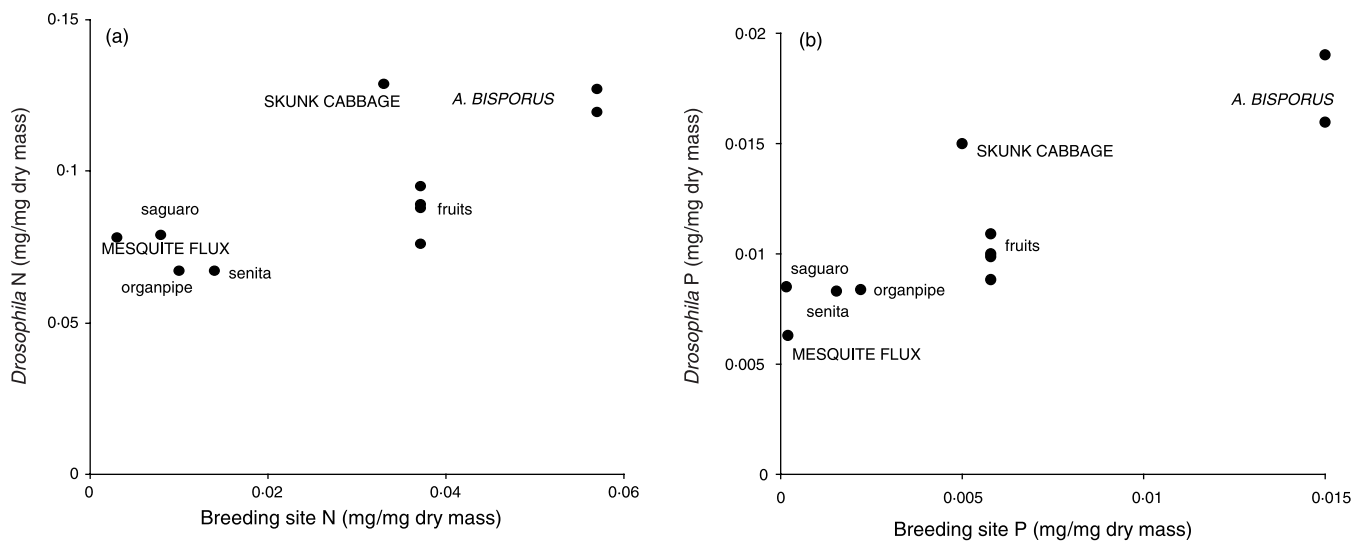


Fig. 2. Correlation between the elemental composition (as percentage of dry mass) of *Drosophila* and their breeding sites: (a) nitrogen; (b) phosphorus. For the frugivorous species of *Drosophila*, the mean N and P content of several fruit types was used (see Markow *et al.* 1999). Data from Markow *et al.* (1999) identified by lower case labels.

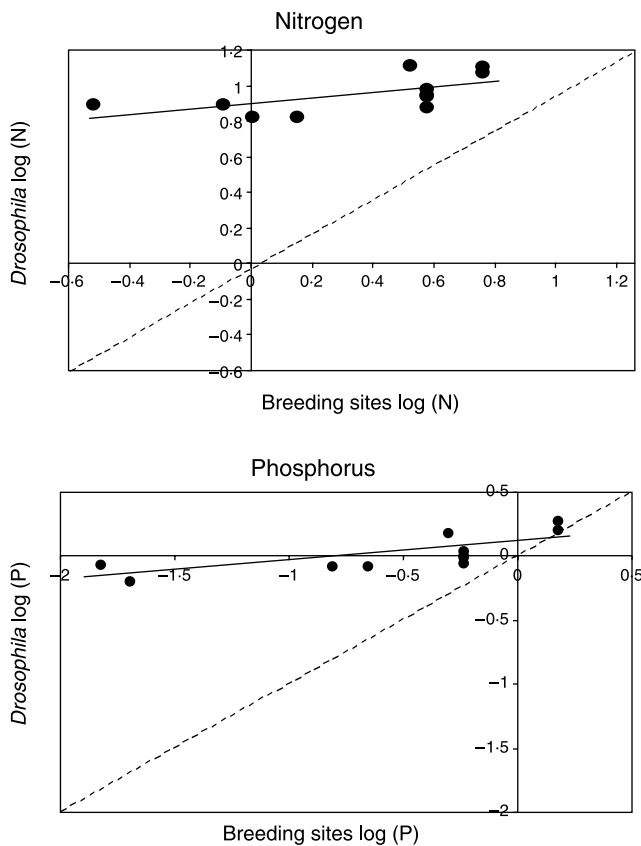


Fig. 3. Nitrogen and phosphorus content of *Drosophila* as a function of that of their resources. Data are percentage of dry mass, plotted on a \log_{10} scale. For the frugivorous species, the mean N and P content of several fruit types was used (see Markow *et al.* 1999). Solid line shows the fitted linear regression. The dashed line (slope = 1, Y intercept = 0) shows the 1 : 1 relationship expected if the elemental composition of the flies equals that of their resources.

for P. To our knowledge this is the first such determination of interspecific stoichiometric constancy.

Finally, the variance in $\log(N : P)$ mass ratios is much greater among the breeding sites than among the different *Drosophila* species ($F_{10,7} = 24.1$, $P < 0.001$; Fig. 4). The slope of this regression is 0.121, with 95% confidence limits from -0.002 to 0.24. Because the slope is significantly less than 1, this shows that the N : P ratio also exhibits significant interspecific constancy across ecologically diverse species of *Drosophila*. Because the slope is positive and almost statistically significant ($P = 0.053$), this indicates that the N : P ratio varies somewhat among *Drosophila* species and thus that the nitrogen and phosphorus contents do not change in perfect proportion across species.

Discussion

The results presented here show that *Drosophila* species vary considerably in their body nutrient contents – by a factor of 3 in the case of phosphorus and by nearly 2 for nitrogen. Although the species we have considered are ecologically diverse, they represent only a small fraction of species in the genus *Drosophila*. It

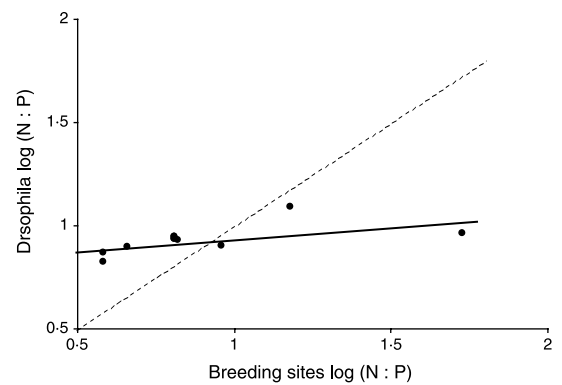


Fig. 4. Nitrogen : phosphorus mass ratios (as percentage of dry mass) of *Drosophila* and their breeding sites. Ratios are plotted on a \log_{10} scale. Solid line shows the fitted linear regression. The dashed line (slope = 1, Y intercept = 0) shows the 1 : 1 relationship expected if the N : P ratio of the flies equals that of their resources.

is therefore likely that the range of elemental composition among other *Drosophila* species is even greater. Our results also show that there is a positive correlation between the elemental composition of a *Drosophila* species and that of the breeding sites where its larvae develop. Consequently, ecologically different categories of flies differ consistently in their elemental content. In terms of both N and P content, the species we have examined can be ranked as mycophagous > frugivorous > cactophilic > mesquite flux breeder, the same rank order as the N and P contents of their breeding sites.

Variation among *Drosophila* species in elemental composition may have important ecological and life-history consequences. For instance, if the fertility of adult flies is limited by inorganic nutrient availability (Sang 1978), then species whose larvae develop in nutrient-rich substrates, and whose adults are therefore nutrient-enriched upon emergence, may be less constrained as adults in their food selection and may be able to reproduce at a younger age. Such nutrient-rich flies may also provide superior environments for organisms that feed on *Drosophila*, such as parasitic wasps and nematodes.

Because the N and P contents of the flies do not appear to level off with increasing concentrations in their resources (Fig. 3), this suggests that N and P are limiting factors over the range of nutrient concentrations that occur naturally in the diverse food resource types of *Drosophila*. Thus, breeding site selection in *Drosophila* species may be governed to an important extent by resource nutrient content.

Stoichiometric interactions between consumers and resources take place within a broader ecological setting that can affect the elemental composition of the resources. It is known, for example, that the nutrient content of leaves varies as a function of a number of environmental variables. The nitrogen content of plants varies among habitat types, being typically low in evergreen shrubs and trees, intermediate in deciduous

chaparral shrubs and old field herbaceous plants, and high in desert herbs (Field & Mooney 1986). A number of environmental variables may contribute to such differences. In both temperate and tropical forests the nutrient contents of leaves are positively correlated with soil fertility (Boerner 1984; Vitousek & Sanford 1986), which in turn can be a function of ecosystem age (Chadwick *et al.* 1999). It has also been found that plants inhabiting colder areas tend to have higher nutrient contents (Korner 1989), that plants sprouting after fire have higher nitrogen and phosphorus contents (Rundel & Parsons 1980), and that many species of plants grown under conditions of elevated CO₂ have lower leaf nitrogen contents (Yin 2002). Thus, global changes in atmospheric CO₂, temperature and fertilization are all likely to have significant effects on the elemental composition of leaves.

In the present study, we found a correlation between the nutrient contents of various types of breeding sites and the *Drosophila* species that utilize these resources. Similar correlations between resource and consumer nutrient content have recently been found in other groups, such as zooplankton (DeMott *et al.* 1998). Given these resource–consumer correlations, it would be interesting to determine if there are correlations between environmental conditions, such as soil fertility, mean annual temperature or ambient CO₂, and the elemental composition of consumers in various habitat types. If the phosphorus content of consumers is affected by such environmental variables, this could have effects on life-history evolution independent of a particular selective regime (Elser *et al.* 2000b). Finally, if nutrient contents are generally correlated from soils to plants to higher trophic levels, then stoichiometric balance may play a role, in addition to energy inputs and dynamical stability, in limiting population densities and the number of trophic levels within ecosystems (Lindeman 1942; Lawton & Pimm 1978; Lawler & Morin 1995; DeMott & Gulati 1999).

One striking finding in the present study was the strong correlation between N and P contents across breeding sites and among *Drosophila* species (Fig. 1). Positive correlations between N and P have also been found in zooplankton species (Hessen & Lyche 1991; Sterner & Hessen 1994). These findings suggest that the N : P ratios of a species or group of consumers (e.g. *Drosophila*, zooplankton) may be relatively closely constrained, perhaps resulting from constraints on protein/nucleic acid ratios (Elser *et al.* 2000b). Consequently, although either N or P might limit the absolute nutrient content of an organism, the close coupling of N and P contents means that low availability of one element brings about generally low organismal levels in both. Because plants are only weakly homeostatic and are capable of storing nutrients in excess of their current needs (Marschner 1995; Sterner & Elser 2002), low availability of one element will have less of an effect on the level of other elements in these organisms.

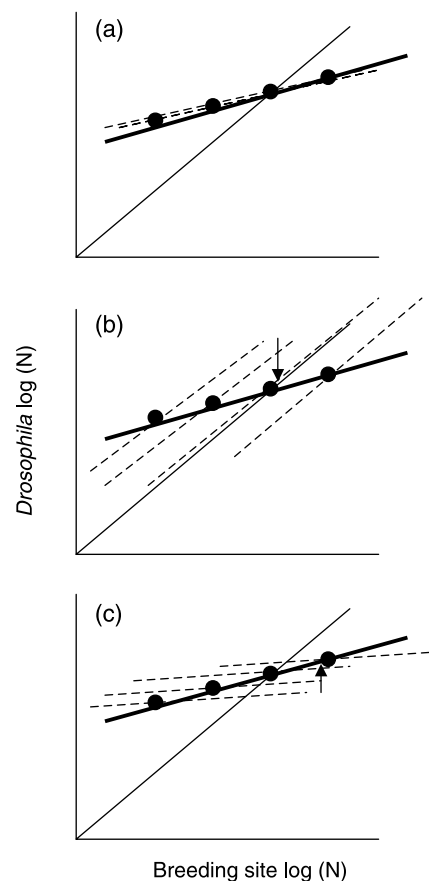


Fig. 5. Models for the manifestation of interspecific constancy in elemental content. This example considers nitrogen content of flies and their resources. The points indicate flies that have developed on their normal breeding sites. Dashed lines indicate intraspecific responses to variation among breeding sites, and the thin solid lines show the relationship expected if the elemental composition of the flies equals that of their resources. Evolutionary responses indicated by arrows. (a) Interspecific constancy = intraspecific homeostasis. Different species have identical responses to variation in resources, so no evolutionary changes are associated with a shift to a new breeding site. (b) Interspecific constancy > intraspecific homeostasis. Species evolve to have lower nutrient content in response to higher nutrient content of resources. (c) Interspecific constancy < intraspecific homeostasis. Species evolve to have higher nutrient content in response to higher nutrient content of resources.

The substantial level of interspecific constancy in body composition among *Drosophila* species raises an important evolutionary question: what is the relation between intraspecific homeostasis and interspecific constancy? We can envision three very different means by which interspecific constancy can be achieved (Fig. 5). First, all *Drosophila* species may exhibit similar homeostatic responses to variation in resource elemental composition: for any given fly species, the slope describing body nutrient content as a function of food nutrient content may closely resemble the overall relationship observed across all fly species (Fig. 5a). In such a case, an evolutionary shift to a new type of breeding site would not entail any concomitant shift in stoichiometric response.

Second, intraspecific homeostasis may be less than that the interspecific constancy of different species on their normal breeding sites: for any given species, the slope describing body nutrient content as a function of food nutrient content may be greater than the overall relationship among fly species (Fig. 5b). The interspecific constancy might arise from stoichiometric constraints on the functional biology of *Drosophila*. In this case, a shift to a new type of breeding site richer in, say, nitrogen would bring about selection to reduce the nitrogen content of flies, in effect countergradient selection.

Finally, and perhaps most likely, homeostasis may be greater within species than interspecific constancy among them: for any given species, the response of its body nutrient content to changes in food quality may be highly restricted (Fig. 5c). This response could arise if there are strong constraints on the elemental composition of any given fly species. A shift to a new type of breeding site might bring about selection for a fly's body composition to more closely match that of its food, i.e. in the opposite direction of that envisioned under the second scenario. Because our data were obtained from various species of *Drosophila* that had developed on their normal breeding sites – mushrooms, fruits, skunk cabbages, cactus necroses or mesquite fluxes – we cannot yet distinguish among these three possibilities. Because the level of intraspecific stoichiometric homeostasis has been found to vary greatly among species, from virtually none ($H = 1$) to essentially absolute ($H = \infty$) (Sternner & Elser 2002), there is no *a priori* reason to rule out any of the three possibilities.

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