

Stable isotope ratios of carbon and nitrogen in natural populations of *Drosophila* species and their hosts

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Summary

1. Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were determined in seven species of wild-caught *Drosophila* (see Patterson & Stone 1952) and their natural hosts in order to assess if any relationships existed between isotope signatures of the flies and their hosts. The species included the cosmopolitan *D. hydei*, *D. arizonae*, *D. simulans* and *D. pseudoobscura* collected from rotting fruit (commercial melons), and the cactophilic *D. nigrospiracula*, *D. mojavensis* and *D. pachea* collected from their specific host plants (Saguaro, Organpipe and Senita Cactus, respectively).

2. Isotope signatures were clearly different among the natural hosts, with fruit and each species of cactus segregating into a non-overlapping pattern on plots of $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$.

3. Wild-caught *Drosophila* exhibited interspecific differences in isotope signatures that reflected the patterns observed for their natural hosts. For most species, values for $\delta^{15}\text{N}$ were 3.0–5.0‰ higher in the flies, in agreement with the expected $\delta^{15}\text{N}$ enrichment with increased trophic level.

4. For *D. nigrospiracula*, changing food resources from the natural host (Saguaro) to a laboratory diet of yeast for only 24 h resulted in a shift in stable isotope signatures toward the values of the new resource.

5. The results suggest that stable isotope analysis can be a valuable tool in studies of resource ecology and feeding habitats in *Drosophila*, and is sensitive enough to detect recent feeding history.

Key-words: Food resources, fruitfly, stable isotopes

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Introduction

Species of the genus *Drosophila* exhibit extreme diversity in their resource ecology. Some species utilize almost any decaying fruit or vegetable material associated with human habitation, while others are highly specialized feeders that, because of specific nutritional requirements, are restricted to a single host species (Carson 1971). Because of their known phylogenetic relationships, *Drosophila* species are well suited for studies on the evolution of niche specialization and its implications for life-history variation. What is missing, however, is definitive information about resource use and requirements of *Drosophila* species in nature.

In a recent comparison of the elemental compositions of several natural *Drosophila* hosts, major differences were detected between commercial melons and cacti in nitrogen (N) and phosphorus (P) levels (Markow *et al.* 1999). Most cosmopolitan species such as *D. melanogaster* and *D. simulans* utilize decaying commercial fruit as

a feeding and breeding site. Because decaying melons are much richer in both N and P than columnar cactus hosts of the *Drosophila* endemic to the Sonoran Desert, the question is raised as to whether these elements limit growth and reproduction in cactophilic species.

When elements such as N or P are insufficient in an organism's typical resource, a number of strategies may be employed to compensate for the limitation (Mattson 1980; Tauber, Tauber & Masaki 1986). Several of these involve direct shifts to other food sources richer in the necessary nutrient. *Drosophila* are small and highly vagile, making it impossible to monitor the activities of an individual fly for more than a few minutes. Therefore, if one is interested in whether flies visit or utilize additional feeding sites, one is dependent upon more inferential methods of investigation. For example, from analysis of crop contents, one can infer with certainty that flies ingest yeast, bacteria and nectar (Starmer 1982). But such analyses may not reveal the source of the items and may miss less visible evidence of utilization of other feeding sites.

A close correspondence between the stable isotope ratios of carbon and of nitrogen between animals and their controlled laboratory diets was reported by

DeNiro & Epstein (1978, 1981). Stable isotope ratios of carbon, $\delta^{13}\text{C}$, were first used by Fry, Joern & Parker (1978) and Boutton, Cameron & Smith (1978) to demonstrate the differential utilization of C_3 vs C_4 plants by a variety of species of grasshoppers. Subsequent studies revealed stable isotope ratios to be sensitive to dietary differences of termites from different castes within the same colony (Boutton, Arshad & Tieszen 1983) and to discriminate among aquatic insect species feeding upon either detritus or plankton in the same lake (Rau 1980). By adding the use of $\delta^{15}\text{N}$, Mihuc & Toetz (1994) were able to produce dual scatter plots ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) that clearly distinguished between the detritus and periphyton diets of different species of alpine aquatic insects. Analysis of $\delta^{13}\text{C}$ in tissues of bats and their food resources allowed Fleming, Nuñez & da Silveira Lobo (1993) to distinguish between bats feeding on C_3 and crassulacean acid metabolism (CAM) plants and provided insights on bat migratory behavior.

We undertook a study of the relationship between the C and N stable isotope signatures of wild *Drosophila* and those of their natural hosts in order to assess *Drosophila* resource ecology. The study was designed to address three specific questions: (1) How much variability exists in the isotope signatures of *Drosophila* hosts? (2) Do wild-caught *Drosophila* exhibit interspecific differences in isotope signatures and, if so, how closely do those differences reflect the signatures of their known feeding sites? (3) Can recent changes in feeding history detectably alter fly isotope signatures? To address these questions, we examined the C and N stable isotope ratios of seven species of *Drosophila*, *D. hydei* Sturtevant, *D. arizonae* Patterson & Wheeler, *D. simulans* Sturtevant, *D. pseudoobscura* Frowla, *D. nigrospiracula* Patterson & Wheeler, *D. mojavensis* Patterson & Crow and *D. pachea* Patterson & Wheeler collected from their natural feeding sites. The observed food material was simultaneously collected and analysed.

Materials and methods

COLLECTION AND PREPARATION OF PLANT MATERIAL SAMPLES

Melons (Cantaloupe, Honeydew and Watermelon) were purchased from a market in Tempe, Arizona, sectioned and placed outdoors at a local residence. A 1-m² patch of decaying melons was maintained throughout autumn 1996 by periodically adding more fruit. When taking samples of necrotic material for analysis, care was taken to remove any immature or adult arthropods. In addition to the necrotic flesh, separate samples of melon rind were taken from the same Watermelon and the same Cantaloupe. Cacti were sampled from the sites where cactophilic *Drosophila* species were collected: Saguaro (*Carnegiea gigantea* Britten & Rose) in the Superstition Mountains of

Arizona, Senita (*Lophocereus schottii* Britten & Rose) from San Carlos and Guaymas, Sonora, Mexico, and Organpipe (*Stenocereus thurberi* Britten & Rose) from San Carlos, Sonora. For Saguaro, tissue samples were taken from five plants, each about 2 km apart. Two of these, Saguaro 1 and Saguaro 2, had active necroses, enabling comparison of healthy and necrotic tissue from the same plant. Two different Senita plants, also separated by distances of approximately 2 km, were sampled. Both Senita plants had necrotic tissue, but a comparison of healthy and necrotic tissue was obtained only on Senita 2. Arthropod-free samples of both necrotic and healthy tissue (flesh only) were taken from the same plants. All samples were thoroughly dried by placing them in an oven at 60 °C for 1 week. Dried samples were then ground to a fine powder with mortar and pestle.

COLLECTION AND PREPARATION OF *DROSOPHILA* SAMPLES

Adult flies were aspirated directly from their feeding sites into vials and taken to the laboratory. Four species, *D. hydei*, *D. arizonae*, *D. simulans* and *D. pseudoobscura*, were collected from the patch of rotting fruit described above. Two of these species, *D. simulans* and *D. hydei*, are considered cosmopolitan, typically associated with human habitation (Patterson & Stone 1952). The other two are considered to be primarily either cactophilic (*D. arizonae*) or slime-flux breeding (*D. pseudoobscura*) but are polyphagic and in urban settings are often found feeding on decaying fruits and vegetables together with the more cosmopolitan species. In this study all four species were considered frugivorous. The remaining three species are exclusively cactophilic and were collected from pockets of necrotic flesh from their specific host cacti found in the Sonoran Desert (Heed 1978). Flies of *D. nigrospiracula* were aspirated from Saguaro in the Superstition Mountains east of Phoenix, Arizona. The other two species, *D. mojavensis* and *D. pachea*, were found on their respective hosts, Organpipe and Senita, in San Carlos, Sonora, Mexico. In the laboratory, males and females of each species were immediately separated and placed in individual glass vials and transferred to an oven at 60 °C for 72 h. Flies, numbering from about 20–200, were pooled in order to obtain enough material for analysis. Dried flies were counted and weighed before being ground to a fine powder with a mortar and pestle.

CARBON AND NITROGEN ANALYSES

The dried powder samples obtained from plant material and flies were analysed for C and N stable isotope composition. In order to assess the homogeneity of the pooled fly samples, and to estimate experimental error due to handling and processing, three subsamples of each sample were taken and analysed separately for stable isotope composition. Each host sample was

analysed in triplicate. Stable isotope ratios are reported as the mean (\pm standard error) of the triplicate determinations. The automated analyses were performed with a Europa Scientific 20/20 mass spectrometer (Europa Scientific, Vondalia, OH). The precision routinely obtained in our laboratory is 0.1‰ or less for both ^{13}C and ^{15}N . Standards were air (nitrogen) and PDB = Pee Dee belemnite (carbon). Stable isotope ratios were calculated as follows: $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) = $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

STATISTICAL ANALYSIS

Statistical comparisons of mean values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in host types (melons, Saguaro, Organpipe and Senita) and *Drosophila* species were conducted using the Kruskal–Wallis test (SYSTAT 7.0.1).

Results

STABLE ISOTOPE RATIOS IN DIETARY RESOURCES

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for natural dietary substrates of the *Drosophila* species used in the present study are shown in Table 1. Values for standard errors given in Table 1 (and Table 2) indicated that measurement error was very small in all samples. When samples of the same host type came from different parts of the same plant (i.e. rind vs flesh for melons and necrosis vs healthy tissue for cacti), values for both $\delta^{13}\text{C}$ and

Table 1. Values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for natural hosts of seven species of *Drosophila*. All values are means (\pm SE) for three separate determinations on each sample

Hosts	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Fruit (hosts for <i>D. hydei</i> , <i>D. arizonae</i> , <i>D. simulans</i> and <i>D. pseudoobscura</i>)		
Cantaloupe rind	-20.5 \pm 0.3	5.5 \pm 0.1
Cantaloupe flesh	-20.8 \pm 0.3	5.4 \pm 0.0
Honeydew (rind and flesh)	-21.2 \pm 0.3	9.9 \pm 0.1
Watermelon skin	-21.6 \pm 0.2	3.8 \pm 0.1
Watermelon flesh	-20.7 \pm 0.2	3.1 \pm 0.1
Saguaro (host for <i>D. nigrospiracula</i>)		
Necrosis (Sample 1)	-11.6 \pm 0.0	1.9 \pm 0.0
Necrosis (Sample 2)	-10.9 \pm 0.2	1.8 \pm 0.0
Healthy tissue (Sample 1)	-11.1 \pm 0.3	1.6 \pm 0.1
Healthy tissue (Sample 2)	-11.0 \pm 0.2	1.0 \pm 0.2
Healthy tissue (Sample 3)	-11.3 \pm 0.3	2.7 \pm 0.1
Healthy tissue (Sample 4)	-10.2 \pm 0.2	1.5 \pm 0.3
Healthy tissue (Sample 5)	-9.5 \pm 0.2	2.1 \pm 0.1
Organpipe (host for <i>D. mojavensis</i>)		
Necrosis	-17.5 \pm 0.2	6.7 \pm 0.5
Healthy tissue	-15.0 \pm 0.2	5.9 \pm 0.5
Senita (host for <i>D. packea</i>)		
Necrosis (Sample 1)	-15.7 \pm 0.2	13.8 \pm 0.1
Necrosis (Sample 2)	-6.0 \pm 0.1	13.7 \pm 0.5
Healthy tissue (Sample 2)	-16.3 \pm 0.1	13.8 \pm 0.3

Table 2. Values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in males and females of seven species of wild-caught *Drosophila*. All values are means (\pm SE) for three separate determinations on each sample

Species	Sex	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
<i>D. hydei</i>	Male	-21.8 \pm 0.2	10.1 \pm 0.1
<i>D. hydei</i>	Female	-20.5 \pm 0.3	10.2 \pm 0.1
<i>D. arizonae</i>	Male	-21.8 \pm 0.4	8.9 \pm 0.2
<i>D. arizonae</i>	Female	-21.2 \pm 0.5	9.0 \pm 0.4
<i>D. simulans</i>	Male	-22.8 \pm 0.5	9.4 \pm 0.4
<i>D. simulans</i>	Female	-22.5 \pm 0.3	8.5 \pm 0.4
<i>D. pseudoobscura</i>	Male	-22.7 \pm 0.2	10.4 \pm 0.0
<i>D. pseudoobscura</i>	Female	-22.7 \pm 0.3	9.5 \pm 0.1
<i>D. nigrospiracula</i>	Male	-11.0 \pm 0.3	6.8 \pm 0.2
<i>D. nigrospiracula</i>	Female	-10.8 \pm 0.3	6.3 \pm 0.2
<i>D. mojavensis</i>	Male	-11.7 \pm 0.1	9.7 \pm 0.1
<i>D. mojavensis</i>	Female	-11.1 \pm 0.4	10.0 \pm 0.1
<i>D. packea</i>	Male	-13.2 \pm 0.2	14.3 \pm 0.2
<i>D. packea</i>	Female	-12.0 \pm 0.6	13.4 \pm 0.2

$\delta^{15}\text{N}$ were very similar (Table 1). Inspection of Table 1 reveals that the within-species isotope signatures for the multiple samples of Saguaro and Senita were similar, even though these samples came from different locations (see first subsection of Materials and methods). Because the frugivorous *Drosophila* species are all associated with all three types of melons, unlike the host-specific cactophilic species, the three types of melons were grouped when comparing stable isotope ratios of the natural hosts statistically. This resulted in four host groups (melons, Saguaro, Organpipe and Senita).

Values for $\delta^{13}\text{C}$ showed significant differences among host types (Kruskal–Wallis test statistic = 11.95, $P = 0.007$, $df = 3$). Columnar cacti showed greater enrichment in ^{13}C (i.e. less negative values of $\delta^{13}\text{C}$) than melons. Mean values (\pm SE) for $\delta^{13}\text{C}$ (‰) were -20.0 (\pm 0.1) for melons ($n = 5$), -10.9 (\pm 0.3) for Saguaro ($n = 7$), -16.2 (-15.0, -17.5; $n = 2$) for Organpipe and 16.0 (\pm 0.2) for Senita ($n = 3$). Values for $\delta^{15}\text{N}$ also differed significantly among host types (Kruskal–Wallis test statistic = 13.99, $P = 0.003$, $df = 3$). Mean values (\pm SE) for $\delta^{15}\text{N}$ (‰) were 5.5 (\pm 1.3) for melons ($n = 5$), 1.8 (\pm 0.4) for Saguaro ($n = 7$), 6.3 (5.9, 6.7; $n = 2$) for Organpipe and 13.8 (\pm 0.1) for Senita ($n = 3$).

A plot of $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ for the natural hosts (Fig. 1) also revealed the segregation of the four groups into a non-overlapping pattern of distinctive stable isotope signatures.

STABLE ISOTOPE RATIOS IN WILD-CAUGHT *DROSOPHILA*

Stable isotope signatures for males and females of each species of *Drosophila* were similar (Table 2). The small values for standard errors given in Table 2 again indicated that measurement error was small in all samples. Nonetheless, the need to pool flies resulted in only a single mean value for males and females of each

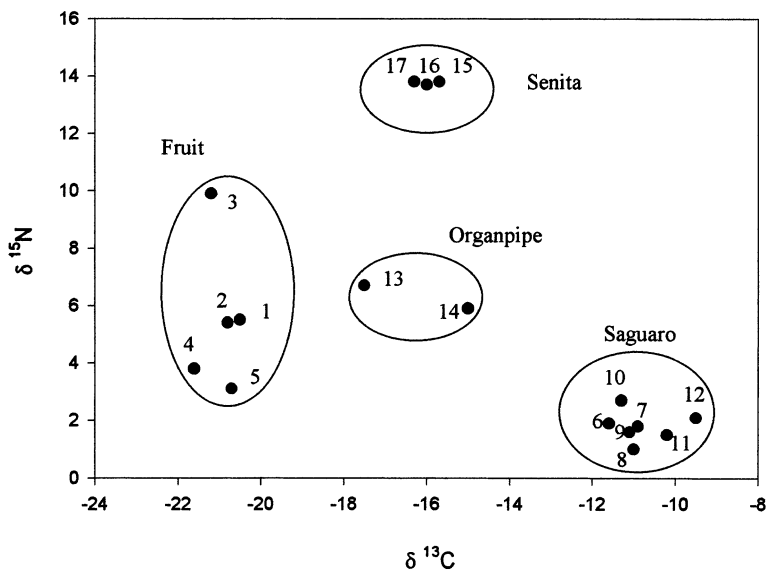


Fig. 1. C and N stable isotope signatures of natural hosts of *Drosophila*. Key to samples: 1 Cantaloupe rind; 2 Cantaloupe flesh; 3 Honeydew (combined rind and flesh); 4 Watermelon skin; 5 Watermelon flesh; 6 Saguaro necrosis (Sample 1); 7 Saguaro necrosis (Sample 2); 8 Saguaro (Sample 1); 9 Saguaro (Sample 2); 10 Saguaro (Sample 3); 11 Saguaro (Sample 4); 12 Saguaro (Sample 5); 13 Organpipe necrosis; 14 Organpipe; 15 Senita necrosis (Sample 1); 16 Senita necrosis (Sample 2); 17 Senita (Sample 2).

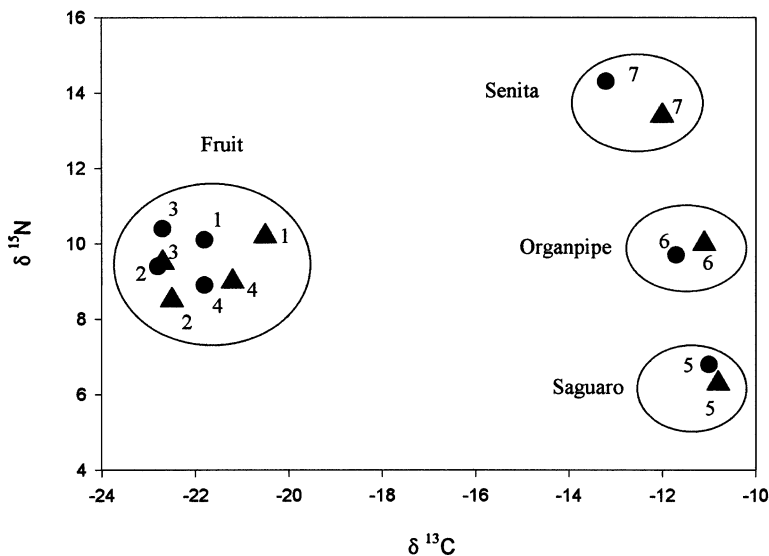


Fig. 2. C and N stable isotope signatures of wild-caught *Drosophila*. Key to species: 1 *D. hydei*; 2 *D. simulans*; 3 *D. pseudoobscura*; 4 *D. arizonae*; 5 *D. nigrospiracula*; 6 *D. mojavensis*; 7 *D. packea*. Circles = male; triangles = female. Natural hosts for each species are shown for comparison with Fig. 1.

species. Because we were interested in determining if there were interspecific differences in stable isotope signatures that might reflect host signatures, the entire data set in Table 2 was analysed with a cluster analysis (SYSTAT 7.0.1; $\alpha = 0.05$). The result gave four groups, each of which contained the males and females of the *Drosophila* species that corresponded to each of the four natural hosts. The four groups are readily appar-

ent in a plot of $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ for males and females of each species (Fig. 2) where the groups reflect the same pattern as seen for the natural hosts (Fig. 1).

To examine whether a short episode of feeding on a different resource with a different stable isotope signature would alter the isotope signature of the flies, the following experiment was conducted. A subset of freshly collected *D. nigrospiracula* was allowed to feed for 24 h on a paste of baker's yeast, *Saccharomyces cerevisiae*, in the laboratory before analysing stable isotope ratios. The untreated flies (Table 2) and the yeast were also analysed. As before, each sample was analysed in triplicate and the standard errors indicated low measurement error. If 24 h of feeding on yeast influenced fly stable isotope ratios, it was predicted that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of yeast-fed flies would become more similar to the isotope ratios of the yeast than those ratios found in the control flies. The mean value (\pm SE) for $\delta^{13}\text{C}$ in males of *D. nigrospiracula* fed on yeast shifted from -11.0‰ for untreated flies to $-14.9 (\pm 0.1)\text{‰}$; females shifted from -10.8‰ to $15.4 (\pm 0.2)\text{‰}$. The values for $\delta^{15}\text{N}$ changed from 6.8 to $4.8 (\pm 0.0)\text{‰}$ in males and 6.3 – $4.6 (\pm 0.0)\text{‰}$ in females. These changes were all in the direction of the isotope ratios for the yeast ($\delta^{13}\text{C} = -15.9 \pm 0.2\text{‰}$; $\delta^{15}\text{N} = 1.2 \pm 0.0\text{‰}$), consistent with the prediction.

Discussion

Our overall interest was in whether *Drosophila* hosts exhibit differences in stable isotope ratios substantial enough to allow inference about intra- and interspecific resource use in nature. As is evident in Fig. 1, this is certainly the case when stable isotope ratios of both C and N are examined simultaneously.

The major source of interspecific variation in host $\delta^{13}\text{C}$ is the photosynthetic pathway used by the plant species under investigation (Smith & Epstein 1971). Plants using the C_3 pathway are characterized by $\delta^{13}\text{C}$ values ranging from -21 to -33‰ , while C_4 plants range from -9 to 17‰ . CAM plants include a broader range of $\delta^{13}\text{C}$ values, between -10 to -30‰ , because of the presence of both the C_3 and C_4 pathways. Columnar cacti of the species studied here are CAM plants (Szarek & Ting 1977), while the melons are produced by C_3 plants. Values for $\delta^{13}\text{C}$ reported here for columnar cacti (-10 to -18‰ ; Table 1) are in good agreement with previously reported values (Mooney, Troughton & Berry 1974; Fleming *et al.* 1993).

Values of $\delta^{15}\text{N}$ in plants are very sensitive to soil variability, especially fertilization practices, which has led to the suggestion that ecological studies using N stable isotopes be conducted in habitats removed from regions of agricultural activity (Peterson & Fry 1987). The Sonoran Desert sites from where our samples were taken are free of this potential problem. Natural variation in soil composition, however, may also contribute to differences in observed $\delta^{15}\text{N}$. The three cactus species we tested showed very different

values for $\delta^{15}\text{N}$. All Saguaro were from Arizona, and while the plants did not come from the same site, the $\delta^{15}\text{N}$ values showed only small variations, from 1.0 to 2.7‰. Furthermore, as with the Saguaro samples, Senita plants from different sites were highly consistent. The $\delta^{15}\text{N}$ value in necrotic tissue of the one Organpipe sampled differed dramatically from the corresponding value from Senita 1, although both cacti were all from the same site (San Carlos) and located only a few metres apart. These observations indicate that plant species, rather than local soil characteristics, underlie the stable isotope ratio differences observed among host species. Another factor influencing $\delta^{15}\text{N}$ is the ability of the plant to fix nitrogen. Nitrogen-fixing plants, legumes, tend to exhibit lower values than non-nitrogen-fixing plants (Mattson 1980). Finally, the lack of any real differences between necrotic and healthy cactus tissues demonstrates that the growth of microbes in the rotting material does not appreciably alter its N isotope ratio.

Our observations suggest that stable isotope ratios of C and N can be used to study diet breadth in cactophilic *Drosophila*. Consumers are usually similar to their hosts for $\delta^{13}\text{C}$ (DeNiro & Epstein 1978); a difference greater than 1.5‰ suggests that another resource is being utilized (Fry *et al.* 1978). Values for body $\delta^{15}\text{N}$ are, however, typically enriched by an average of 3.4‰ (range 1.3–5.3‰) for each successive increase in food chain trophic position (Minagawa & Wada 1984). Therefore, flies would be expected to match their hosts closely for $\delta^{13}\text{C}$, but show substantial enrichment in $\delta^{15}\text{N}$. *Drosophila* using necrotic fruit and cacti, whether in the larval or adult stage, feed upon both plant tissue and the microorganisms involved in the decay process. In terms of trophic level, then, they are simultaneously one and two trophic levels above their plant resources.

In order to assess how closely the stable isotope values of the flies fall into the anticipated ranges of their natural hosts, differences were calculated between the stable isotope value in wild-caught *Drosophila* of each sex and the corresponding mean value of the respective host (Table 3). No major within-species differences were seen between the sexes in either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$. Interspecific comparisons of $\delta^{13}\text{C}$ suggest two patterns. The four frugivorous species, and the cactophilic *D. nigrospiracula*, were generally similar to their respective hosts, with $\delta^{13}\text{C}$ differences ranging from –1.8 to 0.5‰. The other cactophilic species, *D. mojavensis* and *D. pachea*, were enriched in $\delta^{13}\text{C}$ (2.8–5.2‰). Flies of all species, except *D. pachea*, showed an enrichment of $\delta^{15}\text{N}$ ranging from 3.0 to 5.0‰; $\delta^{15}\text{N}$ values in males and females of *D. pachea* were very similar those of the Senita host plant.

It is unclear, in the absence of additional studies, what accounts for the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ observed between *D. mojavensis* and *D. pachea* and their hosts. A number of factors can explain the observed deviations in these predicted relationships (Mihuc &

Table 3. Differences in stable isotope ratios between wild-caught *Drosophila* and the mean value of the respective natural host. Positive values indicate enrichment in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in flies with respect to their host

Species	Sex	Difference in $\delta^{13}\text{C}$ (‰)	Difference in $\delta^{15}\text{N}$ (‰)
<i>D. hydei</i>	Male	–0.8	4.6
<i>D. hyde</i>	Female	0.5	4.7
<i>D. arizonae</i>	Male	–0.8	3.4
<i>D. arizonae</i>	Female	–0.2	3.5
<i>D. simulans</i>	Male	–1.8	3.9
<i>D. simulans</i>	Female	–1.5	3.0
<i>D. pseudoobscura</i>	Male	–1.7	4.9
<i>D. pseudoobscura</i>	Female	–1.7	4.0
<i>D. nigrospiracula</i>	Male	–0.2	5.0
<i>D. nigrospiracula</i>	Female	0.0	4.5
<i>D. mojavensis</i>	Male	4.6	3.4
<i>D. mojavensis</i>	Female	5.2	3.7
<i>D. pachea</i>	Male	2.8	0.5
<i>D. pachea</i>	Female	4.0	–0.4

Toetz 1994) between insects and their food, including the ingestion but not assimilation of N-containing compounds, ingestion of other host material that differs in N but not C stable isotope ratios, and tissue-specific differences in incorporation or elimination of ^{15}N (Gannes, O'Brien & Martinez Del Rio 1997). Because our experiments examined whole flies, this last factor is not likely to contribute to the differences seen. It is conceivable, however, that species of *Drosophila* might differ in assimilation of ^{15}N .

An alternative explanation is that adults of *D. mojavensis* and *D. pachea* utilize additional, and as yet undescribed, resources. Food sources low in N and P are considered to be of poor nutritional quality (Elser, Dobberfuhl, MacKay & Schampel 1996). Cacti have only a small fraction of the N and P found in fruits, even those necrotic cactus portions which contain microbial communities richer in these elements (Markow *et al.* 1999). Variability in element content also exists among cactus species (Markow *et al.* 1999), supporting earlier suggestions that species such as organpipe are of comparatively poorer nutritional quality (Etges & Klassen 1989). Cactophilic *Drosophila* species may have developed compensatory nutrient acquisition strategies such as utilization of other hosts (Breitmeyer & Hocutt 1998; Polak & Markow 1998) or metabolic use of atmospheric elements from volatiles (Etges & Klassen 1989). Our observed departures from expected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are consistent with such alternative nutritional strategies. This explanation assumes, however, that even short feeding episodes at a different source can detectably alter isotope ratios in whole body preparations of *Drosophila*. Our results on *D. nigrospiracula* fed for 24 h on baker's yeast demonstrate that the stable isotope technique is sensitive enough to detect recent dietary shifts provided that hosts differ in their stable isotope ratios.

For some species of *Drosophila*, yeast analysis suggest that diets of larval and adult flies may differ in nature (Carson 1971). The extent to which such differences, when they exist, influence stable isotope ratios should be examined. While we do not know whether adults of the frugivorous species (*D. hydei*, *D. arizonae*, *D. simulans* and *D. pseudoobscura*) analysed here eclosed from the specific substrates on which they were collected, all four species have been reared, on several other occasions, from these same fruit types (T. A. Markow, unpublished data). The close correspondence of stable isotope ratios, especially $\delta^{13}\text{C}$, between host and fly is either a function of recent adult feeding history in these four species, or it reflects a close similarity between larval and adult diet.

This study was not meant to be an exhaustive description of the relationships between host and fly stable isotope ratios. We were interested in whether adequate variation exists among hosts and among wild-caught flies to ask if the approach will be useful in studies of *Drosophila* resource ecology. It clearly does. Furthermore, our preliminary test of whether short-term feeding on a contrasting resource with a different stable isotope signature could be detected in *D. nigrospiracula* suggests that stable isotope techniques may be useful in future studies of *Drosophila* resource use in nature.

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