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# Yeast communities associated with *Drosophila* species and related flies in an eastern oak-pine forest: a comparison with western communities

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## SUMMARY

Intestinal yeast mycobiota were studied in 14 species of *Drosophila* and in the drosophilid species *Chymomyza amoena*, captured at Pinery Provincial Park, Ontario. Over 56 yeast species, some undescribed, were isolated. These yeast communities were compared with those from two similar surveys conducted in western portions of North America. The community structures were influenced significantly by the habitat rather than phylogeny of the flies. Geographic separation was a factor affecting yeast taxa frequencies in the fly species, but it was largely overshadowed by ecological factors when the communities were described physiologically. The notion that habitats are filled by yeasts which add up to a suitable physiological potential, more or less independently of their taxonomic affinities, was thus confirmed.

## INTRODUCTION

The ecological relationships which link decaying plants and molds, yeasts, and various insects were outlined nearly a century ago [3,9]. This work showed that yeasts are maintained and cultured in the body of the insect hosts, the yeasts are a major food source for the hosts, and the hosts readily disperse their yeasts to new substrates to participate in decay. Study of this mutualism was renewed by biologists interested in both yeast and drosophilid ecology [4,13,23,25,27–29]. The degree of mutualism and coevolution in plant–yeast–insect communities remains a question of importance for understanding the evolution and ecology of community members, as well as for understanding decay processes in ecosystems. In this paper, we focus on discriminating between potential influences on the structure of yeast–drosophilid communities in North America.

Several species of *Drosophila*, and the sap fly *Aulacigaster leucopeza* Meigen have been collected in the Yosemite region of California by Phaff and coworkers [22], and the yeasts of their alimentary canal have been studied. Similar investigations have been conducted with *Drosophila* species known to feed or breed in cacti of the southwestern portion of North America [26,28]. Some information exists on the habitat specificity of several eastern North American drosophilid flies [6,7,30]. It thus seemed appropriate to examine the fly–yeast ecology of an eastern North American site comparable to similar sites in the west. Pinery Provincial Park (Lake Huron, Ontario) comprises, among other vegetation types, an oak-pine forest (*Quercus rubra* L. and *Pinus strobus* L.) which may be reviewed, in some ways, as a Great Lakes region counterpart of the oak-fir forest (*Q. kelloggii* (Gord et Glend.) Lindl and *Abies concolor* Nemb.) of the Yosemite region. The community composition of other yeast habitats at Pinery Provincial Park has been studied [5,17].

In this paper, the yeasts associated with drosophilids collected at the Pinery are compared with yeasts from flies of regions in Arizona, California, and Mexico, to assess the respective influences of the phylogeny, ecology, and geography of the flies on the structure of their yeast communities.

## MATERIALS AND METHODS

### *Isolation procedures*

Fermenting banana baits were set in Pinery Provincial Park, near the shore of Lake Huron, Ontario. The attractant was contained in buckets covered with fine nylon netting to prevent contact with the flies. The buckets were secured to the ground with hardware cloth and pegs. Eight baits were distributed over a 200 × 160 m area, and collections were carried out over a 2-day period (July 17 and 18, 1982). Flies were captured by aspiration, and stored in cool containers. They were then anesthetized with ether, identified, surface-sterilized in 70% ethanol, and dissected to expose their crops. The crop contents were then streaked over plates of YM agar (Difco, Detroit, MI, USA) acidified to pH 3.7 with HCl.

### *Yeast identification*

Yeast strains were characterized physiologically by replica plating, and they were identified as recommended by van der Walt and Yarrow [31]. Unidentified isolates were recorded as

This paper is dedicated to Professor Herman Jan Phaff in honor of his 50 years of active research which still continues.

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such or under a generic descriptor, awaiting further characterization.

*Data analysis*

Sampling strategy and data preparation were as described by Lachance and Starmer [19]. The principles and algorithms used in determining deviation probabilities and in principal components analysis were discussed elsewhere [20]. In summary, the physiological responses of the yeasts in a community (defined in this case by a particular fly) were averaged, and the mean community responses were compared to those of all known yeast species [2]. The probability that each community response deviated significantly ($\alpha = 0.05$) from that of a random sample of yeasts was then calculated with reference to a theoretical binomial distribution based on random expectations. The probabilities, given signs which indicate their directionality, were then subjected to multivariate analysis.

Cross-tabulation of data to reveal structure in their frequency distributions was accomplished by generating clustering sequences both for the yeast species (rows) and the fly species (columns), on the basis of their respective inter-correlations. Clustering algorithm LOSIDE was modified to suit this purpose. The data were then rearranged according to the clustering sequences, using algorithm CONTIN.

The multivariate relationships present in the distribution of yeasts in flies were examined by means of reciprocal averaging [14]. Algorithm CA, designed to accomplish this, deviates in some respects from the original method. Certain difficulties are inherent in the method and have to do with the weighting of frequencies and of the canonical variates.

First, the total number of yeasts in each fly depends to a certain extent, but not entirely, on the number of flies per relevé. To analyze frequencies with no concern for sample size would greatly bias the results in favor of fly species which are dominant or captured more easily than others. Conversely, the use of relative frequencies (number of yeasts per fly) would add an undue weight to fly species with very low sample sizes, since, in reciprocal averaging, eigenanalysis is performed on a matrix of deviations from expectations [14]. A useful solution was to divide the yeast frequencies by the square root of fly sample size, a compromise between two extremes.

A second modification to Hill's [14] procedure was in ranging canonical variate scores. It was felt that Hill's suggestion to range the scores from zero to their associated eigenvalue may in some way obscure unusually sharp trends present in a particular set of variates. Instead, the unranged scores were multiplied by their corresponding eigenvalue. The result is the ability to juxtapose any two sets of independent variates while taking into account their reciprocal proportionality. The programs LOSIDE, CONTIN, and CA were written in Fortran from commonly available algorithms (Lachance, unpublished).

## RESULTS AND DISCUSSION

*Isolations*

The *Drosophila* species considered in this study are listed in Table 1, along with their suspected habitats. *Drosophila algonquin, D. affinis, D. athabasca, D. robusta, D. falleni* and *D. melanica* were isolated at the Pinery in relatively high numbers. Other species recovered only occasionally were *D. melanogaster, D. busckii, D. putrida*, and *D. hydei*. Rare isolates of *D. quinaria, D. recens*, and *D. testacea*, also captured at the Pinery, yielded no yeasts and were not considered further. In addition, a number of specimens of *Chymomyza amoena* Loew, a drosophilid fly associated with bleeding trees and with fruits [1], were successfully dissected.

The species *D. nigrospiracula, D. pachea, D. mojavensis*, and *D. mettleri* are cactophilic flies [11,12] from which yeasts were recovered by Starmer et al. [26]. The remaining species in Table 1 were studied for their yeast mycobiota by Phaff et al. [22], in forests of the Sierra Nevada, in the Yosemite region of California. Two specimens of *D. busckii* were also reported in the latter survey, along with many representatives of *Aulacigaster leucopeza* Meigen, a non-drosophilid sap-feeding fly.

The yeasts recovered from Pinery flies are listed in Table 2. Members of Nadsonioideae (*Hanseniaspora* and *Saccharomycodes* spp.) and of their anamorphic counterpart (*Kloeckera* spp.) were most abundant (53 isolates), followed by species of *Kluyveromyces* (45 isolates). Moderate numbers (33) of *Saccharomyces sensu lato* (*Saccharomyces, Torulaspora*, and *Zygosaccharomyces* spp.) were also present. Other common isolates were *Debaryomyces hansenii*, and species of the genera *Pichia, Metschnikowia*, and *Candida*.

Some isolates were not identified as members of previously described species. Beside a heterogeneous group of atypical *Candida* species, there were four rather similar strains of haplobiontic *Pichia*, four atypical *Saccharomyces*, and three diazonium blue positive yeasts, two of which adopted an apiculate habit under certain conditions.

*Comparison of different relevés of yeasts from North-American flies*

*(A) Flies and their yeast taxa distribution.* The distribution of yeasts isolated from Pinery, Yosemite, and cactus flies is shown in Table 3. Note that the frequencies are arranged in order to display group structure, as a result of bidimensional clustering. Without any formal analysis, it is easily seen that the cactus-colonizing flies form a discrete group, with little yeast species overlap with forest flies. The cactus fly communities also stand out as the most specific, as indicated by their low effective numbers of species [15] and their high yeast/fly ratios.

Yosemite flies belonging to the *obscura* group are very homogeneous in their yeast composition, and in turn, they greatly resemble *Aulacigaster* spp. and *D. pinicola* on the same basis (Table 3). Too few yeasts were recovered from *D. miranda*, also in the *obscura* group, to allow a significant comparison here. Many yeast taxa are shared between these and our eastern isolates of the *obscura* group. *Hanseniaspora* and related genera, *Kluyveromyces* species, and members of *Saccharomyces sensu lato* show much overlap among eastern and western isolates. Considerable overlap also exists with *D. melanica* and *D. robusta*. The Pinery flies generally exhibited a higher degree of yeast species diversity compared with the

TABLE 1

Summary of classification and suspected habitats of *Drosophila* species considered in this study

| Subgenus | Group | Species (abbreviation) | References on habitats | | | |
|---|---|---|---|---|---|---|
| | | | Fruits and decaying plants | Fungi | Sap or flux | Cactus rots |
| Sophophora | melanogaster | *D. melanogaster* Meigen (Mg) | [7,21,30] | [30] | [30] | |
| | obscura | *D. pseudoobscura* Frolova (Ps) | | | [30] | |
| | | *D. persimilis* Dobzhansky et Epling (Pe) | | | [30] | |
| | | *D. miranda* Dobzhansky (Mi) | | | [30] | |
| | | *D. algonquin* Sturtevant et Dobzhansky (Al) | | | [30] | |
| | | *D. affinis* Sturtevant (Af) | [7] | | [7,30] | |
| | | *D. athabasca* Sturtevant et Dobzhansky (At) | [7] | [7] | [7,30] | |
| | | *D. azteca* Sturtevant et Dobzhansky (Az) | | | [30] | |
| Dorsilopha | busckii | *D. busckii* Coquillett (Bu) | [7,21,30] | | | |
| Drosophila | funebris | *D. subfunebris* Stalker et Spencer (Su) | [30] | | | |
| | robusta | *D. robusta* Sturtevant (Ro) | | [7] | [7,21,30] | |
| | pinicola | *D. pinicola* Sturtevant (Pi) | | [24,30] | [21] | |
| | quinaria | *D. quinaria* Loew (Qu) | [7] | [21,30] | | |
| | | *D. recens* Wheeler (Re) | | [21,30] | | |
| | | *D. falleni* Wheeler (Fa) | | [21,30] | | |
| | | *D. occidentalis* Spencer (Oc) | | [30] | [21] | |
| | testacea | *D. testacea* Roser (Te) | | [21,30] | | |
| | | *D. putrida* Sturtevant (Pu) | | [7,21,30] | [7] | |
| | melanica | *D. melanica* Sturtevant (Mn) | [7] | | [7,21,30] | |
| | repleta | *D. californica* Sturtevant (Ca) | | | [21,30] | |
| | | *D. nigrospiracula* Patterson et Wheeler (Ni) | [21] | | | [11,12] |
| | | *D. hydei* Sturtevant (Hy) | [7,21,30] | | | |
| | | *D. mojavensis* Patterson (Mo) | | | | [11,12] |
| | | *D. mettleri* Heed (Mt) | | | | [11,12] |
| | nannoptera | *D. pachea* Patterson et Wheeler (Pa) | | | | [11,12] |

western communities, even though the latter were collected over a wider geographic area.

The remaining relevés (Table 3) are generally characterized either by low numbers of flies, or by low yields of yeasts, and so their yeast species distributions are less revealing. Note that in these cases, the usefulness of the effective number of species as an ecological parameter is lost, as it tends to merge with the actual number of species, or even with the total number of strains isolated.

The patterns in Table 3 were formally analyzed by means of reciprocal averaging. As expected, the overwhelming uniqueness of the cactus communities virtually obliterated any remaining patterns, and so no useful information could be obtained other than the obvious.

The same analysis was therefore performed on data from which the cactus relevés had been deleted. The results are shown in Fig. 1. Along the first set of canonical variates (Y1) the communities associated with *C. amoena, D. subfunebris, D. falleni, D. occidentalis*, and *A. leucopeza* emerged, an indication of the occurrence, in those communities, of yeasts not generally present in other flies (Fig. 1(A)). The second axis (Y2) shows the communities as a continuum.

The significance of the patterns in connection with phylogenetic, ecological, and geographic aspects of the flies may be visualized in Fig. 1(B,C,D). In Fig. 1(B), the drosophilid phylogeny proposed by Throckmorton [30] has been superimposed onto the coordinates in Fig. 1(A). These patterns now reveal, along the first axis, a distinction between taxa in the *immigrans-Hirtodrosophila* radiation, and those of the subgenus *Sophophora*. Taxa in the *virilis-repleta* radiation show no such polarity.

Fig. 1(C) is an analogous diagram based on the principal habitats of the flies. Flies associated with decaying plants and with fungi are intermingled, but as a whole they appear distinct from arboreal communities in their responses on both axes. *Chymomyza amoena* is known to associate both with trees and with fruits, and the habitat of this particular sample may be inferred.

Fig. 1(D) reveals that the second axis is tied to a definite geographic gradient opposing eastern (high scores) and western (low scores) flies. This east-west gradient is apparent in both subgenera *Sophophora* and *Drosophila*. The absence of a major discontinuity along the second canonical variable is also indicative of the presence of several yeast taxa shared by many fly species as well.

It should be pointed out that the ordination pictured in

TABLE 2

Taxonomic designations and total frequencies (F) of yeasts isolated from flies at Pinery Provincial Park, Ontario, Canada

| Taxonomic designation | F |
|---|---|
| *Candida:* | |
| *C. bombicola* (Spencer, Gorin et Tulloch) Meyer et Yarrow | 1 |
| *C. butryi* Nakase | 1 |
| *C. catenulata* Diddens et Lodder | 1 |
| *C. curiosa* Komagata et Nakase | 1 |
| *C. famata* (Lodder) Meyer et Yarrow | 2 |
| *C. karawaiewi* (Jurzitza) Meyer et Yarrow | 2 |
| *C. lactis-condensi* (Lodder et Kregar-van Rij) Meyer et Yarrow | 2 |
| *C. parapsilosis* (Ashford) Langeron et Talice | 1 |
| *C. sake* (Saito et Ota) van Uden et Buckley | 9 |
| *C. stellata* (Lodder) Meyer et Yarrow | 8 |
| *C. tropicalis* (Castellani) Berkhout | 1 |
| *C. valida* (Leberle) van Uden et Buckley | 14 |
| *C. vinaria* Ohara, Nonomura et Yunome ex Smith | 4 |
| *C. vini* (Desmazieres ex Lodder) van Uden et Buckley | 1 |
| *Candida* spp. | 10 |
| *Citeromyces matritensis* (Santa Maria) Santa Maria | 1 |
| *Cryptococcus:* | |
| *Cr. albidus* (Saito) Skinner | 4 |
| *Cr. laurentii* (Kufferath) Skinner | 3 |
| *Cr. luteolus* (Saito) Skinner | 2 |
| *Cr. magnus* (Lodder et Kreger-van Rij) Baptist et Kurtzman | 2 |
| *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij | 11 |
| *Geotrichum penicillatum* (do Carmo Sousa) von Arx | 1 |
| *Hanseniaspora:* | |
| *H'spora vineae* v.d. Walt et Ischeuschner | 11 |
| *H'spora uvarum* | 12 |
| *Kloeckera:* | |
| *Kl. apiculata* (Reess emend. Kloecker) Janke | 28 |
| *Kl. apis* Lavie ex Smith, Simione et Meyer | 1 |
| *Kl. corticis* (Kloecker) Janke | 1 |
| *Kluyveromyces:* | |
| *K. dobzhanskii* (Shehata, Mrak et Phaff) v.d. Walt | 7 |
| *K. lactis* var. *drosophilarum* (Shehata, Mrak et Phaff) Sidenberg et Lachance | 10 |
| *K. thermotolerans* (Phillippov) Yarrow | 25 |
| *K. waltii* Komada | 3 |
| *Metschnikowia:* | |
| *M. pulcherrima* Pitt et Miller | 12 |
| *M. reukaufii* Pitt et Miller | 2 |
| *Phialophora* Meddlar spp. (melanic yeast-like fungi) | 2 |
| *Pichia:* | |
| *P. membranaefaciens* Hansen | 8 |
| *P. pinus* (Holst) Phaff | 3 |
| *Pichia* spp. | 4 |
| *Rhodotorula:* | |
| *Rh. glutinis* (Fres.) Harrison | 3 |
| *Rh. graminis* di Menna | 2 |
| *Rh. minuta* (Saito) Harrison | 3 |
| *Rhodotorula* sp. | 1 |

TABLE 2 continued

| Taxonomic designation | F |
|---|---|
| *Saccharomyces:* | |
| *Sacch. cerevisiae* Hansen | 7 |
| *Sacch. dairensis* Naganishi | 1 |
| *Sacch. kluyveri* Phaff, Miller et Shifrine | 3 |
| *Saccharomyces* spp. | 4 |
| *Saccharomycodes ludwigii* Hansen | 1 |
| *Schizosaccharomyces japonicus* Yukawa et Maki | 3 |
| *Sporobolomyces roseus* Kluyver et van Niel | 1 |
| *Trichosporon cutaneum* (de Baurm., Gougerot et Vaucher) Ota | 1 |
| *Torulaspora delbrueckii* Lindner | 9 |
| *Williopsis:* | |
| *W. californica* (Lodder) Krasilnikov (= *Hansenula californica*) | 1 |
| *W. mrakii* (Wickerham) Krasilnikov (= *Hansenula mrakii*) | 2 |
| *Zygosaccharomyces:* | |
| *Z'sacch. fermentati* Naganishi | 8 |
| *Z'sacch. florentinus* Castelli et Kudrjawzev | 1 |
| Unidentified yeasts | 3 |

Fig. 1 is only a representation of some 20% of the variance in the fly/yeast frequency distribution. This suggests that the yeast species patterns are, for the most part, rather weak, in other words, that the data structure is 'dilute'. This was of course not true when cactus communities were included. For this reason, and in view of the adjustment performed on the frequencies (see Materials and Methods), reciprocal averaging is used here strictly as an exploratory method, and we make no attempt to exploit its parametric capabilities.

*(B) Prevalent yeast taxa.* Figure 2 is a representation of the results discussed above, showing the more important ($n \geqslant 8$) yeast taxa recovered from forest flies at the Pinery and in Yosemite. The impact of apiculate yeasts and of species of *Saccharomyces* and related genera is evident. Numerically important yeasts responsible for the east-west gradient (Y2) are *D. hansenii, M. pulcherrima, T'spora delbrueckii* and two *Candida* species at the eastern end, as opposed to *C. krusei, Kl. corticis, Z'sacch. fermentati* (syn. *Sacch. montanus*), *Sacch. cerevisiae, P. angusta*, and *K. drosophilarum* at the western end. With the exception of *D. hansenii*, a polyphagic yeast species, and of *C. valida* and *Kl. corticis*, nutritionally limited species, those yeasts are physiologically very similar. Overall, they are strong fermenters, and they utilize a moderate number of compounds belonging to relatively similar chemical classes.

Figure 2 also suggests that a rarer yeast species accounts for a large portion of the trends depicted in Fig. 2. The possible synonymy of many yeast taxa imposes limits on further interpretation of their distribution.

*(C) Community physiological profiles.* It is now established that physiological profiles are reliable indicators of ecological [25] and evolutionary [19] aspects of yeast community structure, free of constraints imposed by yeast nomenclatural difficulties. In a comparison of geographically distinct communities associated with tree exudates, the description of yeast mycobiota by the mean vectors of physiological responses was shown to be independent of geographic influences as well [5]. A refinement to this approach [20] was aimed at also eliminating constraints inherent to the biochemical nature of physiological responses in yeasts.

The results presented in Fig. 3 were thus obtained by deriving, for the yeast communities of the flies in Table 3, vectors of probability of deviation from randomly distributed yeasts. These vectors were subjected to principal components analysis, and a large portion of the variation (75.5%) could be reduced to two sets of coordinates. The same method applied to data exclusive of cactophilic flies gave very similar results, and so the latter are included in the discussion to follow.

Figure 3(A) shows the ordination of all fly communities from which at least two yeasts were recovered (minimum sample size for this type of analysis). The geographic trend elicited among forest flies by reciprocal averaging is no longer detectable, as indicated by the diagram in Fig. 3(D). The second axis (Y2) does single out the southern communities, but this separation is equally well accounted for by the flies' habitats, as shown in Fig. 3(C). The first component (Y1) has minimal bearing on the cactus/forest habitat dichotomy prevalent in the yeast taxa clusterings, but instead it polarizes arboreal flies (low scores) against fungus feeders (high scores), with cactus

TABLE 3

Yeasts isolated from *Drosophila* spp. and related flies. Data are from Starmer et al. [26], Phaff et al. [22] and from this study. Taxonomic designations used by Phaff et al. [22] are shown in parentheses. Abbreviations for fly species are given in Table 1

| Yeast species | Frequency of isolation from fly species: | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mt | Ni | Mo | Pa | Az | Ps | Pg | Pe | Au | Pi | Af | Ag | At | Mn | Ro | Al | Bu | Fa | Mg | Oc | Mi | Hy | Bu | Ca | Su | Co | Pu |
| *Candida bombicola* | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | |
| *Candida* spp. | | | | | | | | | | | | 4 | | | | | | | | | | | | | | | |
| *Saccharomyces dairensis* | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | |
| *Saccharomyces* spp. | | | | | | | | | | | | 4 | | | | | | | | | | | | | | | |
| *Candida butyri* | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| *Candida vini* | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| *Candida* sp. | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| *Citeromyces matritensis* | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| *Williopsis californica* | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| *Williopsis mrakii* | | | | | | | | | | | 1 | 1 | | | | | | | | | | | | | | | |
| *Kluyveromyces waltii* | | | | | | | | | | | 2 | 1 | | | | | | | | | | | | | | | |
| Unidentified yeast | | | | | | | | | | | 1 | 1 | | | | | | | | | | | | | | | |
| *Candida valida* (= *C. mycoderma*) | | | | | 1 | | | | | | 6 | 3 | 2 | | | | | 1 | 1 | | 1 | 1 | | | | | |
| *Debaryomyces hansenii* | | | | | | | | | | | 4 | 4 | | 1 | | | | | 1 | | | | | | | | 1 |
| *Metschnikowia reukaufii* | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | 1 |
| *Metschnikowia pulcherrima* | | | | | | | | | | | 4 | 4 | | | | | | 3 | 1 | | | | | | | | |
| *Candida karawaiewi* | | | | | | | | | | | 1 | | 1 | | | | | | | | | | | | | | |
| *Candida sake* | | | | | | | | | | | 3 | 2 | 2 | | | 1 | | 1 | | | | | | | | | |
| *Rhodotorula graminis* | | | | | | | | | | | | | 2 | | | | | | | | | | | | | | |
| *Candida famata* | | | | | | | | | | | 1 | | | | 1 | | | | | | | | | | | | |
| *Cryptococcus luteolus* | | | | | | | | | | | 1 | | | | 1 | | | | | | | | | | | | |
| *Phialophora* spp. | | | | | | | | | | | 1 | | | | 1 | | | | | | | | | | | | |
| *Torulaspora delbrueckii* | | | | | | | | | | | 4 | 1 | | | 4 | | | | | | | | | | | | |
| *Candida* spp. | | | | | | | | | | | | | | | 2 | | | | | | | | | | | | |
| *Saccharomycodes ludwigii* | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | |
| *Pichia pinus* (= *S. pini*) | | | | | | | | | 1 | 1 | | 1 | | | 2 | | | | | | | | | | | | |
| *Phialophora* spp. | | | | | | | | | | | 1 | | | | 1 | | | | | | | | | | | | |
| *Torulaspora delbrueckii* | | | | | | | | | | | 4 | 1 | | | 4 | | | | | | | | | | | | |
| *Candida* spp. | | | | | | | | | | | | | | | 2 | | | | | | | | | | | | |
| *Saccharomycodes ludwigii* | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | |
| *Pichia pinus* (= *S. pini*) | | | | | | | | | 1 | 1 | | 1 | | | 2 | | | | | | | | | | | | |
| *Pichia membranaefaciens* | | | | | | | | | 1 | | 3 | | 2 | | 3 | | | | | | | | | | | | |
| *Schizosaccharomyces japonicus* | | | | | | | | | | | 1 | | 1 | | 1 | | | | | | | | | | | | |
| *Hanseniaspora osmophila/vineae* | | | | | | | 1 | 1 | 2 | 2 | 2 | 1 | | 1 | 4 | 1 | | 1 | 1 | | | | | | | | |
| *Hanseniaspora uvarum* | | | | | | | 1 | 3 | 1 | | 3 | 1 | 3 | 1 | 1 | 1 | | 1 | | | | | | | | 1 | |
| *Kloeckera apiculata* | | | | | | 1 | 5 | 6 | 3 | 1 | 5 | 7 | 9 | 4 | 2 | | | 1 | | | | | | | | | |
| *Kluyveromyces lactis* var. *drosophilarum* | | | | | 2 | 3 | 5 | 1 | 5 | 2 | | 2 | | 2 | 5 | 1 | | | | | 1 | | | | | | |
| *Kluyveromyces dobzhanskii* | | | | | | | | 1 | | | 1 | 2 | | 1 | 1 | 2 | | | | | | | | | | | |
| *Saccharomyces kluyveri* | | | | | | | | | | 1 | | | | | 2 | 1 | | | | | | | | | | | |
| *Rhodotorula glutinis* | | | | | | | | | | 1 | | 1 | | | 1 | 1 | | | | | | | | | | | |
| *Rhodotorula minuta* | | | | | | | | | | 1 | | 2 | | | | | | 1 | | | | | | | | | |
| *Candida stellata* (= *T. stellata*) | | | | | 2 | | | 2 | | 4 | 4 | | 2 | | 2 | | | | | | 1 | | 1 | | | | |
| *Candida lactis-condensi* | | | | | | | | | | | 1 | | | 1 | | | | | | | | | | | | | |
| *Candida* sp. | | | | | | | | | | | 1 | | | 1 | | | | | | | | | | | | | |
| *Hansenual* sp. | | | | | | | | | | | | 2 | | 2 | | | | | | | | | | | | | |
| *Candida krusei* | | | | | 1 | 1 | 2 | 1 | 1 | 2 | | | | | | | | | | 1 | | | | | | | |
| *Pichia angusta* (= *H. angusta*) | | | | | 1 | 2 | 2 | 3 | 5 | 3 | | | | | | | | | | 4 | 1 | | | | | | |
| *Kloeckera corticis* (= *K. magna*) | | | | | 2 | 3 | 7 | 2 | 2 | 1 | 1 | | | | | | | | | | 1 | | | | | | |
| *Kluyveromyces thermotolerans* (= *S. veronae*) | 3 | 3 | | | 3 | 2 | 11 | 8 | 10 | 2 | 6 | 3 | 3 | 1 | 2 | 4 | 1 | 4 | 1 | 2 | | | | | | | |
| *Saccharomyces cerevisiae* (= *S. uvarum*) | | | | | 5 | 5 | 9 | 4 | 3 | 4 | 1 | 1 | | | 1 | | | 4 | | | | | | 1 | | | |
| *Zygosaccharomyces fermentati* (= *S. montanus*) | | | | | 8 | 12 | 15 | 6 | 5 | 3 | 6 | 2 | | | | | | | | 1 | | | | | | | |
| *Candida guilliermondii* | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | |
| *Cryptococcus laurentii* var. *laurentii* | | | | | | | | | 1 | | | | | | | | | 1 | | 1 | | | | | | | |

TABLE 3 continued

| Yeast species | Frequency of isolation from fly species: | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mt | Ni | Mo | Pa | Az | Ps | Pg | Pe | Au | Pi | Af | Ag | At | Mn | Ro | Al | Bu | Fa | Mg | Oc | Mi | Hy | Bu | Ca | Su | Co | Pu |
| *Pichia fermentans* | | | | | | | 1 | 1 | | 1 | | | | | | | | | | | | | | | | | |
| *Trichosporon cutaneum* (= *O. lactis*) | | | | | | | 1 | 1 | | | | | | | | | | 1 | | | | | | | | | |
| *Cryptococcus magnus* | | | | | | | | | | | | | | | | | 1 | 1 | | | | | | | | | |
| *Rhodotorula* sp. | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | |
| *Zygosaccharomyces florentinus* | | | | | | 1 | 1 | 1 | | | | | | | | | 1 | | | | | | | | | | |
| *Pichia pastoris* (= *S. pastori*) | | | | | | 2 | 2 | | | | | | | | | | | | | | | | | | | | |
| *Candida colliculosa* | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | |
| *Candida inconspicua* | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | |
| *Geotrichum fermentans* (= *T. fermentans*) | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | |
| *Hanseniaspora valbyensis* | | | | | | | | | | 6 | | | | | | | | | | | | | | | | | |
| *Kluyveromyces wickerhamii* | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | |
| *Aciculoconidium aculeatum* | | | | | 1 | | | | | | 1 | | | | | | | | | | | | | | | | |
| *Candida glabrata* | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | |
| *Candida* sp. | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | |
| *Candida catenulata* | | | | | | | | | | | 1 | | | 1 | | | | | | 5 | | | | | | | |
| *Candida parapsilosis* | | | | | | | | | | | 1 | 1 | | | | | | | | 1 | | | | | | | |
| *Cryptococcus albidus* var. *diffluens* | 3 | | | | | | | | | 1 | | | | | | | | | 1 | 1 | | | | | | 1 | 1 |
| *Rhodotorula rubra* (= *R. mucilaginosa*) | 1 | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| *Candida curiosa* | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| *Cryptococcus laurentii* var. *flavus* (= *R. aurea*) | | | | | | | | | 1 | | | | | | | | 1 | | | | | | | | 1 | | |
| *Candida vinaria* | | | | | | | | | | | | 1 | 1 | | | | | 1 | 1 | | | | | | | | |
| *Candida tropicalis* | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | |
| *Sporobolomyces roseus* | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | |
| Unidentified yeast | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | |
| *Crytococcus albidus* var. *albidus* | | | 1 | 1 | | | | | | | | | | | | | | | 2 | | | | | | | | |
| *Pichia heedii* | 3 | 6 | | 25 | | | | | | | | | | | | | | | | | | | | | | | |
| *Sporopachydermia cereana* | | 1 | 1 | 12 | | | | | | | | | | | | | | | | | | | | | | | |
| *Candida ingens* | | | 5 | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| *Cryptococcus sonorensis* | 1 | | 17 | | | | | | | | | | | | | | | | | | | | | | | | |
| *Candida spp.* | | | 14 | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pichia amethionina* var. *amethionina* | | | 20 | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pichia kluyveri* | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pichia mexicana* | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pichia cactophila* | 17 | 23 | 37 | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pichia amethionina* var. *pachycereana* | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Candida mesenterica* | | | | | | | 1 | 1 | | | | | | | | | | | | | 1 | | | 1 | | | |
| *Pichia toletana* (= *P. xylosa*) | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | |
| *Candida* sp. | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | |
| *G. penicillatum* | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | |
| *Kloeckera apis* | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | |
| Total | 28 | 34 | 97 | 71 | 26 | 32 | 64 | 42 | 42 | 44 | 71 | 54 | 28 | 15 | 37 | 15 | 2 | 28 | 8 | 18 | 7 | 1 | 1 | 2 | 1 | 4 | 2 |
| Effective number of species | 3 | 2 | 4 | 2 | 6 | 5 | 7 | 10 | 9 | 16 | 21 | 18 | 6 | 7 | 15 | 8 | 2 | 13 | 8 | 6 | 7 | 1 | 1 | 2 | 1 | 4 | 2 |
| Number of flies sampled | 30 | 36 | 60 | 28 | 35 | 38 | 75 | 44 | 30 | 103 | 61 | 78 | 24 | 8 | 16 | 16 | 1 | 53 | 5 | 56 | 14 | 1 | 2 | 3 | 2 | 13 | 9 |

and plant decay habitats in between. The groupings in terms of *Drosophila* phylogeny (Fig. 3(B)) shows considerably more overlap. In particular, the cases of ecological divergence represented, the sap feeding versus the cactus feeding *virilis-repleta* and sap feeding versus fungus-feeding *immigrans-Hirtodrosophila*, result in grouping according to habitat similarity rather than phylogenetic affinity. While *D. pinicola* of the *Hirtodrosophila* group has been reared from mushrooms [24], the adults have been collected from sap flows. Since we are studying adult ecology we have grouped *D. pinicola* with the sap feeders.

The main factors underlying the first component (Fig. 3) were growth on ribose, D-arabinose, melibiose, starch, and rhamnose ($r > 0.8$), and to a lesser degree, growth on erythritol, lactose, ribitol, galactitol, and L-arabinose ($r > 0.7$). Many of these traits are characteristic of polyphagic or generalistic yeasts such as *Debaryomyces* or *Cryptococcus* species. One would suspect that communities so defined (i.e. the fungus communities) are associated with habitats in which nutrients are diverse or dilute.

The second component (Y2, Fig. 3) clearly isolates the cactus flies from the rest, and outlines the specialized nature of

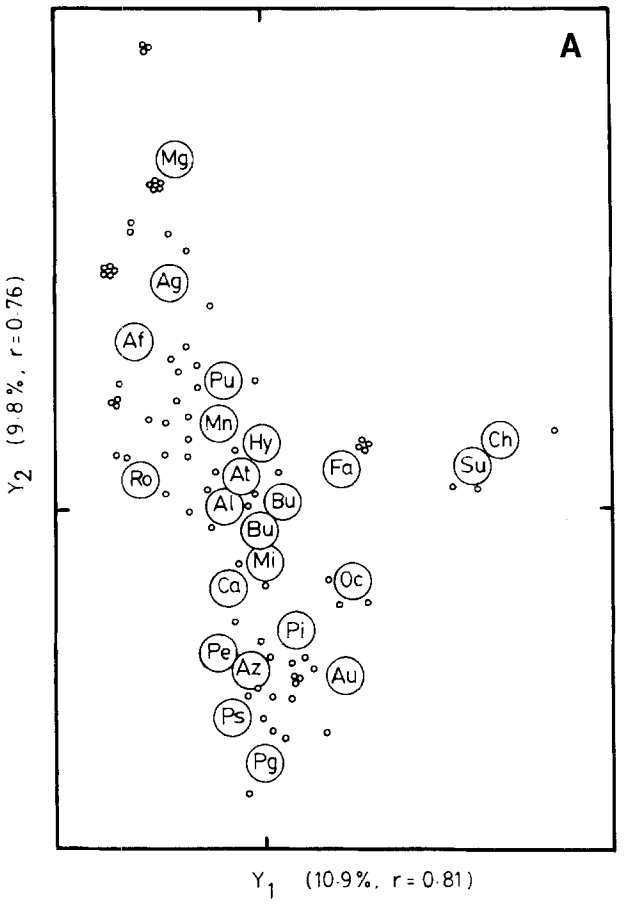


Fig. 1. Ordination of yeast communities associated with fly species, based on the first two sets of canonical variates of their frequency distribution. (A) The flies represented are *Aulacigaster leucopeza* (Au), *Chymomyza amoena* (Ch), *Drosophila affinis* (Af), *D. algonquin* (Al), *D. athabasca* (At), females of the affinis group (Ag), *D. azteca* (Az), *D. busckii* (Bu), *D. californica* (Ca), *D. falleni* (Fa), *D. hydei* (Hy), *D. melanica* (Mn), *D. melanogaster* (Mg), *D. miranda* (Mi), *D. occidentalis* (Oc), *D. persimilis* (Pr), *D. pseudoobscura* (Ps), flies of the persimilis-pseudoobscura group (Pg), *D. pinicola* (Pi), *D. putrida* (Pu), *D. robusta* (Ro), and *D. subfunebris* (Su). The yeast species are represented by open circles. Tic marks on axes indicate the location of the origin. (B) Proposed phylogenetic groupings of drosophilids [30] are shown, superimposed on the fly species coordinates. (C) Grouping of fly coordinates on the basis of predominant habitats (Table 1). (D) Geographic grouping of fly coordinates.

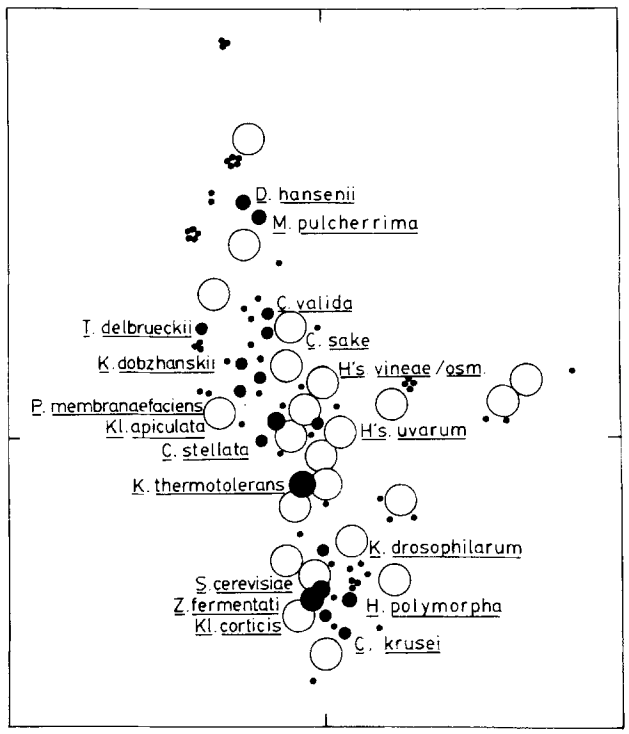


Fig. 2. Ordination of yeasts recovered from fly species, redrawn from Fig. 1. Yeast species are shown as solid circles commensurate to the number of individuals recovered for each species. Numerically important species ($n \geqslant 8$) are labelled. Fly coordinates are shown as open circles.

cactophilic flies as yeast habitats. This second set of coordinates was positively correlated with the use of methyl-$\alpha$-glucoside, maltose, melezitose, sucrose, inulin and raffinose ($r > 0.8$), and negatively correlated with lactic acid and ethanol utilization ($r < -0.8$), a pattern already elucidated in yeast communities of the cacti themselves [20].

The third component, not represented in Fig. 3, accounted for little variation (6.5%), and was moderately correlated ($r = 0.70$) with growth at 37 °C, a trait which may be viewed as linked to geographic variation.

The physiological structure outlined above may also be visualized as in Table 4, where only the scores which exhibited significant deviations ($\alpha = 0.05$) are represented. This tabulation restates the fact that, in general, fly yeast communities utilize fewer compounds than expected from random communities. As already indicated by principal components analysis, communities of predominantly tree and fungus colonizing flies tend to utilize glucosides and fructosides more than normal, while cactus fly communities favor organic acids and ethanol. The geographic trend linked to growth at 37 °C is also evident.

Table 4 also lists the physiological specificity coefficients S [20] associated with each community. In general, cactus fly communities may be considered highly specific, while moderate degrees of specificity are detected in sap fly yeasts.

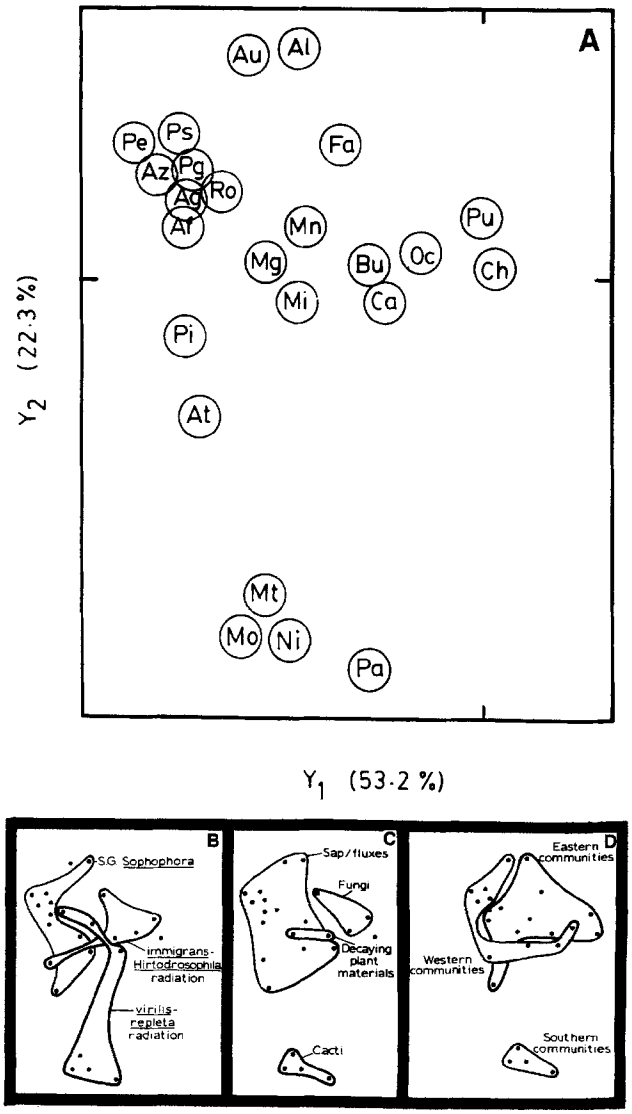


Fig. 3. Ordination of yeast communities associated with flies based on the deviation probabilities of their physiological profiles. (A) The first two principal components are shown. The origin is identified by tic marks. The flies represented are *Drosophila mettleri* (Mt), *D. mojavensis* (Mo), *D. nigrospiracula* (Ni), and *D. pachea* (Pa), in addition to most flies listed in the legend to Fig. 1. Superimposition diagrams (B,C,D) are as described in the legend to Fig. 1.

## GENERALIZATIONS AND CONCLUSIONS

In analyzing three major *Drosophila* yeast collections for major trends among the North American *Drosophila*–yeast communities, we have found that ecological and geographic factors appear to have predominant roles in structuring the communities. *Drosophila* phylogeny does not appear to be a major factor. The effect of geographic variation in yeast taxa clustering may, on the one hand, be due to evolutionary divergence among eastern and western components of the *Drosophila* subgenera. But the consistent geographic gradient occurs in unrelated *Drosophila taxa*, as well as representatives of two other genera. This argues that yeast taxa diverged over the east-west gradient first, followed by similar radiation in the insects. Geographic trends similar to those described here were observed among the yeast taxa associated with tree exudates [18]. In that case also, the trends disappeared when physiology was examined. It is interesting to note that several of the physiological factors found important in distinguishing yeasts from the tree exudates of the study were also important in the arboreal-fungal *Drosophila* distinction reported here.

Along with geography, habitat distinguishes the communities clustered on yeast taxa, cacti being the most distinct, while arboreal and fungal communities also separate well. This arboreal-fungal distinction appears to be the largest factor when considering yeast physiology, with cacti intermediate, and the interpretation of physiological profiles suggests adaptation of fungal communities to a more diffuse source of nutrients. The arboreal-fungal distinction also occurs as a higher bacterial content in fungal communities [10].

*Drosophila* phylogeny is associated with habitat to some extent: several sophophorans are sap feeders, several *immigrans* are fungus feeders, and several repletas are cactus feeders. Each of these groups, however, have members that utilize other habitats [30]. Although the data we present are limited in this respect, there are two examples of habitat shifts within phylogenetic groups. The *virilis-repleta* radiation contains sap feeders (*D. californica, D. melanica*, and *D. robusta*) and cactus feeders (*D. mojavensis, D. nigrospiracula, D. mettleri* and *D. pachea*) while the *immigrans-Hirtodrosophila* radiation has sap/fruit feeders (*D. pinicola* and *D. busckii*) and fungus feeders (*D. falleni, D. putrida* and *D. occidentalis*). In both cases, the *Drosophila* taxa group best with presumed habitat rather than phylogeny. Thus we tentatively conclude that habitat is the major factor, of the three, in determining yeast community physiological profiles.

The primacy of habitat type in determining yeast–*Drosophila* community structure has been reported also by Lachaise et al. [16] in a survey of African savanna-forest ecosystems. Starmer [25] discusses the evolution of *Drosophila* in terms of a step-wise invasion of related habitats. Whether yeasts preceded the insects into new habitats or were vectored by and co-radiated with *Drosophila* is not known. However, the evidence of geographic divergence for yeast taxa, consistent over the insect taxa as discussed above, suggests that the yeasts were the primary invaders, followed by *Drosophila*. The yeast taxa 'available' to flies in different habitats and at different ends of the continent thus appear to differ. This is particularly true when considering the rarer yeasts. The geographic effect which occurs for yeast taxa but not yeast physiology suggests a historical (chance) component, which is reasonable since dispersal abilities of the yeasts are limited and may be primarily a function of the vector insects' dispersal ability [8,10].

Our study does not emphasize the relationships between fly microbiota and that of suspected feeding sites in the Pinery ecosystem. This will be the object of further studies. Some studies on yeast composition of the digestive tract of arboreal flies have not been able to equate directly the yeast composition of tree exudates with that of the flies [4]. Preliminary comparisons indicate that the Pinery collection also will show such inequality. This inequality is in contrast to the cactus–yeast–*Drosophila* communities where cactus microbiota are

TABLE 4

Physiological responses of yeast communities associated with flies, and which differ significantly ($\alpha = 0.05$) from expectations. The number of flies per sample (Nf), the number of yeasts recovered (Ny) and the specificity coefficient (S) are shown

| | Fermentation | Salicin | Cellobiose | Inulin | Raffinose | Sucrose | Methyl-glucoside | Melezitose | Maltose | Starch | Trehalose | Sorbose | Galactose | Lactose | Melibiose | Rhamnose | Ribose | L-arabinose | D-arabinose | Xylose | Glycerol | Erythritol | Inositol | Mannitol | Glucitol | Ribitol | Galactitol | Nitrate | Ethanol | Lactic acid | Citric acid | Succinic acid | 37 °C growth | Nf | Ny | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *D. melanogaster* | | | | | | | | | | | | | | | | | | | | | | − | | | | | | | | | | | 5 | 8 | 0.03 |
| *D. busckii* (P) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 2 | 0.00 |
| *C. amoena* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 13 | 4 | 0.00 |
| *A. leucopeza* | + | | + | + | + | + | + | + | − | | | | − | − | | | − | | − | − | | − | | | | | | | − | | + | | 30 | 39 | 0.46 |
| *D. persimilis* | + | | + | + | + | + | + | + | − | | | | − | − | − | − | − | − | − | − | | − | | − | | | | − | − | | | | 44 | 37 | 0.61 |
| *D. pseudoobscura* | + | | + | + | | + | + | | − | | | | − | − | − | − | | − | − | − | − | − | | | − | − | | | − | − | + | + | 38 | 37 | 0.61 |
| *D. pseudo.* group | + | | + | + | | + | + | | − | | | | − | − | − | − | − | − | − | − | − | − | | − | − | − | − | − | − | − | | + | 75 | 43 | 0.73 |
| *D. azteca* | + | | + | + | + | + | + | | − | | | | − | − | − | − | | − | − | − | − | − | | | | | | − | − | | | + | 35 | 27 | 0.55 |
| *D. athabasca* | | − | | | | | | | − | − | | | − | − | − | − | − | | − | − | − | − | − | − | − | | | − | − | | | | 24 | 21 | 0.52 |
| *D. algonquin* | | | | | + | | + | | | | | | + | | | | | | | − | | − | − | | | | | | | − | | | 16 | 14 | 0.21 |
| *D. affinis* | | | | | | | | | | − | − | | | | − | − | − | − | | − | − | − | − | | − | − | | | − | − | | | 61 | 43 | 0.42 |
| *D. affinis* group | | | | | | | | | | − | | | | − | − | | | | | − | | − | − | − | − | | | | − | − | | | 78 | 43 | 0.30 |
| *D. miranda* | | | | | | | | | | | | | | | | | | − | | | | | − | | | | | | | | | | 14 | 7 | 0.06 |
| *D. robusta* | | | | | + | | | | | − | | | | | | | − | − | | − | − | | − | | − | − | | | − | − | | | 16 | 35 | 0.33 |
| *D. melanica* | | | | | | | | | | | | | | | | | | | | | | | − | | | | | | | | | | 8 | 13 | 0.03 |
| *D. californica* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | 2 | 0.00 |
| *D. pinicola* | + | | | | | | | | | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | | | − | − | | | 103 | 43 | 0.61 |
| *D. falleni* | | | | | | | | + | | | | | | | | | | | | | | | − | | | | | | − | − | | | 53 | 28 | 0.12 |
| *D. occidentalis* | | − | | | | | | | | | | | | | | | | | | | | | − | | | | | | | − | + | | + | 57 | 17 | 0.12 |
| *D. putrida* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 9 | 2 | 0.00 |
| *D. mettleri* | − | − | − | | − | − | − | − | − | − | − | − | − | − | | − | − | − | | − | − | | − | − | − | − | | | | + | + | + | + | 30 | 28 | 0.79 |
| *D. nigrospiracula* | − | − | − | | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | | − | − | − | − | − | − | − | + | + | + | + | + | 36 | 34 | 0.94 |
| *D. mojavensis* | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | | | − | − | − | − | − | − | + | + | | + | + | 60 | 43 | 0.91 |
| *D. pachea* | − | − | − | − | − | − | − | − | − | | − | | − | − | − | − | − | − | | + | + | | | − | − | − | − | − | + | + | | + | + | 28 | 41 | 0.82 |

well characterized and equate with yeasts carried by the *Drosophila* spp. [8,26]. The inequality seen for arboreal flies may only indicate that more work needs to be done to find their natural substrates. The possibility remains, however, that the primary habitat of these yeasts is the *Drosophila* species themselves. As was demonstrated nearly a century ago [9], yeasts will continue to multiply in the crop of insects if the insects feed on sugars. *Drosophila* yeasts may subsist on the nutrients of ephemeral resources which the flies are well suited to forage, such as one-day sap flows from leaf and tree surfaces.

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