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Wineries, *Drosophila*, Alcohol, and *Adh*

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Summary. Previous workers (McKenzie and Parsons, 1972, 1974; McKenzie, 1974; Briscoe et al., 1975) have found anomalous distributions of species of *Drosophila*, of sexes of *D. melanogaster*, and of *Adh* alleles in and around wineries in Australia and Spain. Field studies in California's Sonoma Valley provide evidence that the explanations advanced for these distributions may be incorrect. The anomalous distribution of species was attributed to alcohol, either as a selective agent or as a behavioral stimulus. We find a virtually identical species distribution in the absence of environmental alcohol. The anomalous sex ratio was attributed to differential survival of the sexes when raised on alcohol. We present crude evidence that the difference may simply be a behavioral response to some product of fermentation, which need not be alcohol. Finally, the allele frequency difference reported from Spain was attributed to differential adult mortality on alcohol. We do not find an allele frequency difference even when alcohol is exposed, and therefore suggest that selection is occurring in pre-adult stages.

1. Introduction

The distributions in and around wineries of *Drosophila* species and of alcohol dehydrogenase (*Adh*) alleles in *D. melanogaster* have been the subject of much recent work carried out in Australia and in Spain. We present data, the relevant observations in and around California wineries, that extend and test some of the ideas from this earlier work.

In Australia, McKenzie and Parsons (1972) discovered that, despite the presence of *D. melanogaster* and *D. simulans* outside a winery, only *D. melanogaster* was found inside. This distributional difference was attributed by McKenzie and Parsons to differential alcohol tolerance, and by McKenzie (1974) to a difference in behavioral response to "alcohol fumes." McKenzie (1974) reported an excess of females in the winery and attributed it to an increased mortality of males on alcohol. McKenzie and Parsons

(1974) reported increased alcohol tolerance in lines founded from flies caught inside the winery, but found no difference in *Adh* allele frequencies when comparing flies caught inside with those caught outside.

In Spain, Briscoe et al. (1975) found a difference in allele frequency at the *Adh* locus; the fast allele (*Adh*^F), with higher activity on ethanol (Rasmuson et al., 1966), being in very high frequency inside. They suggest that "adult mortality in the presence of ethanol-rich food plays a major role in maintaining a predominance of the high activity *Adh*^F allele in wine cellar populations."

The wineries of California offer an opportunity for an independent critical look at the ideas advanced by earlier workers. We will present data on the spatial distribution of adult flies, by species, sex, and allelic composition at the *Adh* locus in the case of *D. melanogaster*, and also on breeding sites, by species.

2. Materials and Methods

We have collected flies at various times in and around wineries in Sonoma County, California: primarily at the Gundlach-Bundschu winery in Vineburg, but also at the Sebastiani Winery barrel warehouse in Sonoma, and at the Dry Creek Winery in Healdsburg. Collections were made inside the wineries themselves and in surrounding vineyards and woodlands. One collection was made in an oak woodland in Glen Ellen, near Jack London State Park, more than a mile from the nearest winery. *Adh* genotypes were determined using the methods of Ayala et al. (1972).

At the wineries, after a fermentation has progressed for some time, the wine is pressed off the grape skins and seeds (pomace). Pressing usually reduces the moisture content of the pomace to less than 5% by weight. In California, this pomace is spread in the vineyards as mulch, and is undoubtedly the source for the large numbers of *D. melanogaster* found inside these wineries in the fall. In November, 1977, to determine which species were breeding therein, a two liter sample of pomace from the vineyards adjacent to Gundlach-Bundschu was taken back to the laboratory and the flies raised out of it collected and counted. For contrast, we have information on flies reared from a sample of fallen apples, peaches, and plums collected in Apple Hill, California in El Dorado county (Coyne and Bundgaard, pers. comm.).

In addition, we have performed a crude behavioral experiment: 30 flies of a given species and sex were placed in a rectangular plastic box, and filter paper moistened with fermenting wine must or with a 14% sucrose solution placed at opposite ends. Every hour for 5 h the flies resting on the filter paper were counted and the boxes rotated to randomize any light cues. This experiment was performed four times: separately for males and females of both *D. melanogaster* and *D. simulans*.

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Table 1. Data from collections from outside wineries for the indicated dates. Data are the fraction of the total collection represented by the indicated sex and species or species group. For collections at the Gundlach-Bundschu winery, state of crush indicates whether collection was made before, during, or after the crushing of grapes at the winery

Location date State of crush	Glen Ellen	Dry Creek	Gundlach-Bundschu		
	10/76	10/76	10/76 during	11/76 after	11/77 before
Melanogaster/ simulans ♀♀	0.458	0.689	0.541	0.167	0.168
Melanogaster ♂♂	0.141	0.117	0.201	0.053	0.053
Simulans ♂♂	0.073	0.056	0.066	0.108	0.114
Obscura type ♀♀	0.153	0.049	0.099	0.462	0.302
Pseudoobscura/ persimilis ♂♂	0.158	0.019	0.066	0.181	0.330
Azteca ♂♂	0.000	0.000	0.000	0.012	0.009
Hydei	0.000	0.000	0.004	0.002	0.000
Immigrans	0.017	0.029	0.024	0.013	0.014
Pinicola	0.000	0.000	0.000	0.003	0.010
Total number of flies scored	177	103	543	1079	1048

Table 2. Allelic frequency data and sex ratios (male/female) of flies collected inside and outside the Gundlach-Bundschu winery. The column labelled "open fermenter" indicates whether the collection was before, during, or after the use of an unsealed fermenter. *N* is the total number of individuals run.

Date and Place	Open fermenter	<i>f</i> (<i>Adh</i> ^F)		<i>N</i>	Sex ratio
		In	Out		
10/76 Sebastiani		0.605		57	0.81
Glen Ellen			0.632	91	0.47
Dry Creek		0.606		90	0.44
Gundlach	during	0.596		66	0.19
			0.671	73	0.60
11/76 Gundlach	after	0.586		133	0.38
			0.585	390	0.49
11/77 Gundlach	before	0.679		39	0.41
			n.d.		0.99
	during	0.583		42	0.13
11/78 Gundlach	during	0.597		71	0.17
10/79 Gundlach	during	0.510 ^a		129	n.d.
			0.610 ^a	235	n.d.

n.d. = not determined

^a These allele frequencies are for males only, and differ at the 5% level. Data from McDonald et al. (1980)

3. Results

3.1. Species distribution. Inside each winery, in every collection made, we collected only very large numbers of *D. melanogaster* and an occasional *D. immigrans*. Outside the winery we regularly collected seven or eight species. Data from the outside collections are given in Table 1.

3.2. *Adh* allele frequencies and sex ratios. Frequencies of the *Adh*^F allele inside and outside the wineries are given in Table 2. Also shown in this table are the sample sizes and status of an

Table 3. Median (and range) of numbers of flies on indicated attractant. See text for a more complete description of the experiment

	Melanogaster		Simulans	
	♀♀	♂♂	♀♀	♂♂
Fermenting must	8 (5-15)	3 (2-4)	3 (2-4)	2 (1-3)
Sucrose	1 (0-5)	2 (0-4)	0 (0-4)	2 (1-4)

open fermenter at the Gundlach-Bundschu winery. The column on the far right gives the sex ratios for each collection.

3.3. Flies reared from the pomace. From the two liter sample of pomace brought back to the laboratory, we reared 767 *D. melanogaster* males, 7 *D. simulans* males, 2 *D. azteca* males, 2 *D. pseudoobscura* or *D. persimilis* males and 5 *obscura* group females. *Melanogaster* group females were not counted, but were approximately as frequent as males. From the fallen fruit from Apple Hill, Coyne and Bundgaard (pers. comm.) reared 515 flies, of which 20% were *D. simulans*. In contrast, of the 106 flies caught with sweep nets in the same area, only 1% were *D. simulans*.

3.4. Behavioral experiment. Shown in Table 3 are the results from the behavioral experiment. Tabled are the median and range of numbers of flies of indicated species and sex counted on each substrate. Mortality was negligible during the course of the experiment. Five counts were made in each experiment, but are not independent, as the flies were only somewhat disturbed each hour.

4. Discussion

The several earlier studies discussed above have all related the phenomenon being studied (species distribution, sex ratio, allele frequency distribution) to environmental alcohol. Certain differences in wine-making practice in California allow a critical look at these ideas.

California's wineries differ from their Australian and Spanish counterparts in one striking respect: wines are almost never physically exposed. Thus, we have been unable to find any larvae or pupae inside either Gundlach-Bundschu or Dry Creek, though a single leaking sherry cask in Sebastiani's warehouse provided a very minimal food source for pre-adult stages. The only exception to the policy of never leaving wine exposed is that because of space limitations, Gundlach-Bundschu used a small (several hundred cubic feet) open top fermenter for a single lot of red wine each year from 1976 to 1979. Except when this open fermenter was in use, both Dry Creek and Gundlach-Bundschu fogged the buildings every night with a pyrethrin-based insecticide to keep the number of *Drosophila* in the winery as low as possible. We consider it very unlikely, therefore, that flies are breeding inside either of these wineries.

McKenzie and Parsons (1972) discovered in Australian wineries that, despite the presence of *D. melanogaster* and *D. simulans* outside the winery, only *D. melanogaster* was found inside. We have observed the same phenomenon. Inside the winery we collected very large numbers of *D. melanogaster* and an occasional *D. immigrans*. Outside the winery, as shown in Table 1, we regularly collected seven or eight species, and in many instances *D. melanogaster* was not the most common of these. We extend the observations of McKenzie and Parsons in two ways. First, we have found that *D. pseudoobscura*, *D. persimilis*, *D. azteca*, and *D. hydei*, though fairly common outside, are not found inside the

winery. Second, a few *D. immigrans* have been collected inside the winery, in numbers sufficient to suggest that they disperse passively with respect to the winery. That is, they are approximately as dense (in space) inside the winery as they are outside.

The distributional difference of *D. melanogaster* and *D. simulans* has been explained as being due to differential alcohol tolerance (McKenzie and Parsons, 1972) or due to a difference in behavioral response to "alcohol fumes" (McKenzie 1974). Our data indicate that neither reason can strictly be correct. We find virtually 100% *D. melanogaster* inside the winery even in the absence of environmental alcohol: the inside vs. outside collections of November, 1976, made at Gundlach-Bundschu after all fermentations had been covered, and those of October, 1976, in Sebastiani's barrel warehouse (no fermenters in the building), and at Dry Creek (fermenters outside). The flies cannot encounter winery-produced alcohol as a food source, and probably do not even encounter fermentation fumes at Dry Creek. Furthermore, a collection made in early September, 1977, before any fermentations had begun, yielded only *D. melanogaster*. At this time all the wine was tightly capped so that virtually no environmental alcohol was available. Although the distributional difference of these two species is unequivocal and striking, we find no reason to believe that the cause of this distribution is environmental alcohol.

We regard the filtering out of *D. simulans* at the entrance to the wineries as being perhaps a very artificial behavioral manifestation of the way *D. melanogaster* and *D. simulans*, two sympatric sibling species, parcel the local habitat. Alcohol fumes aside, this observed behavior need not be olfactory in nature. The winery could simply be a dark place. However, the fact that we reared virtually 100% *D. melanogaster* from the pomace suggests that *D. melanogaster* may be more attracted to the fermentation products of grapes than is *D. simulans*. By comparison, at baited traps in the adjacent oak woodland, *D. simulans* males outnumbered *D. melanogaster* males better than 2 to 1 (Table 1), while inside the winery not one of the several thousand males checked were *D. simulans*. Of course, we do not argue that *D. melanogaster* as a species is grape specific, but rather that for some reason, *D. simulans* avoids this locally abundant resource in favor of some other evidently abundant resource. Parenthetically, we note also that the pomace data demonstrate that the cooccurrence of adults of closely related species is not sufficient for the inference of competition in nature.

In another locality, our collection records suggest, much less dramatically, that we have identified a resource which *D. simulans* prefers over *D. melanogaster*. 21% of the flies reared from fallen fruit from Apple Hill were *D. simulans*, while *D. simulans* constituted only 1% of the adult population in that location. Of course, these data could be explained in other ways, but it is possible that in this locality, *D. melanogaster* was using some unidentified additional resource.

McKenzie (1974) attributes the excess of females in the winery to a higher mortality of males on alcohol. We suggest that this may alternatively be due to a behavioral difference. The results of the behavioral experiment reported in Table 3 are a crude test of this hypothesis. No differences in the numbers of individuals resting on either end were found for *D. simulans* or for *D. melanogaster* males. However, significantly more *D. melanogaster* females were found on the wine must than on the sucrose.

It is not necessary that alcohol be the behavioral stimulus for the species or for the sexes. (It is also not necessary that alcohol be the selective agent, if any.) An earlier behavioral experiment similar to the one reported failed to detect any difference in attractiveness of a 10% ethanol solution versus sucrose for

either species for either sex. The fumes produced during fermentation consist of many components, the most common of which is CO₂. Alcohols constitute only a small fraction of the gasses emitted. Acetic acid, acetaldehyde, and several other volatile aromatic compounds are also liberated in small amounts during wine fermentation (Amerine and Joslyn 1970, pp. 349ff). Though the importance of the various compounds with respect to survival or as behavioral cues cannot be evaluated as mole fraction, there is no particular reason to regard alcohols as the most important component. Various substances have been found to be better attractants of *Drosophila* than is ethanol. Barrows (1907) found a mixture of ethanol and acetic acid to be most attractive, and Hutner et al. (1937), testing 150 compounds, found diacetyl (2,3-butadione), acetaldehyde, indol, and ethyl acetate best. That adults may also be conditioned by volatile components in their larval environment (Thorpe 1939) is also important in this respect. Further study is needed on this aspect of the problem.

In addition to the species distribution and the sex ratio, the allelic composition of the population at the *Adh* locus is of interest. Briscoe et al. attribute the allele frequency difference they find to differential adult mortality. If this is true, then when an open fermenter is active, we would expect to see the same thing in California wineries. (The *Adh^F* and *Adh^S* alleles found in California are probably the same as those found in Spain; Kreitman (1980) demonstrated a lack of variability within electromorphs at this locus.) The data in Table 2 demonstrate that the only allele frequency difference found is in fact the wrong way—the *Adh^F* allele is in higher frequency *outside* in 1979. The most important consideration here may be that Spanish wineries support a resident fly population, while their California counterparts do not. Briscoe et al. report collecting large numbers of larvae and pupae from the tops of open containers of wine. This suggests that the difference observed by Briscoe et al. is more likely to result from differential *pre*-adult fitnesses. If this is the case, it explains why we did not find the predicted allele frequency differences.

However, a global synthesis of the relationship between *Drosophila* and wineries remains elusive, because of the findings of McKenzie and Parsons (1974): in their wineries there is a resident population, and flies derived from this population exhibit increased tolerance of alcohol, even though the *Adh^F* allele frequency is the same inside and outside the winery. It would be interesting to know if the difference in alcohol tolerance of flies with different *Adh* genotypes (Gibson 1970) still exists within the Australian winery strains. Perhaps the Spanish and Australian populations have found different ways to accomplish the same adaptation.

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Erratum

In the article entitled, “The Analysis of Contact Sampling Data” by P. deJong, L.W. Aarssen and R. Turkington, published in volume 45, 1980, pp. 322–324, there are four numbers (1), (2), (3), (4) in the text which do not refer to the respectively numbered equations. These were designated in the galley proof to represent the location for insertion of four statements before publication, but the numbers instead of the statements were printed. The statements that were to appear are as follows:

At (1), in the last paragraph of the introduction, insert: The basic difference from looking at association in quadrat sampling is that with contact sampling, only two species can be present in any one sample.

At (2), in the left column on p. 323, insert: In general, there will be serious overestimation of the expected number of joint occurrences.

At (3), in the final paragraph of the paper, insert: ... and may raise difficulties in some vegetation types when being forced in the field to decide where one individual ends and another starts.

At (4), in the final paragraph of the paper, insert: There is no reason to presume any ecological distinction between these ordered pairs in view of the contact sampling scheme.

Erratum

In the article, “Influence of Litterbags on Growth of Fungal Vegetative Structures,” by T.V. St. John, published in Volume 46, No. 1, 1980, pp 130–132, the sentence beginning on the sixth line of the results section (p. 131) should read: “The mean numbers of structures in each treatment were no litter bag: 5.3, coarse mesh: 2.7, fine mesh: 2.5.”