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Enzyme Polymorphism and Species Discrimination in Fruit Flies of the Genus *Dacus* (Tephritidae)

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Abstract

Sympatric populations of *D. tryoni* and *D. neohumeralis* are difficult to completely distinguish taxonomically. Using five pigmentation characters, each of some taxonomic value, a small proportion of individuals cannot be assigned to either species nor definitely classified as hybrids. To aid in species discrimination and hybrid identification gene frequencies in natural populations were estimated at three polymorphic protein loci, an alcohol dehydrogenase (Adh), an octanol dehydrogenase (Odh) and an esterase (E-2). Samples of flies were taken from four sites spread over 1200 miles along the Australian eastern coast.

Within each species allelic frequencies at each locus were largely the same at all localities. Consistent differences in gene frequencies between species occurred at all three loci, strongly supporting the hypothesis of two distinct gene pools. The Adh locus best discriminated between species with a unique allele occurring in *D. neohumeralis* at a frequency of 0·85. None of the loci showed complete differentiation and hence it was not possible to find a quick and easy method to distinguish the species nor to detect field hybrids.

Directional selection of laboratory populations for a change in callus colour (the best pigmentation character for separating the species) indicated that at the Adh and E-2 loci frequencies of major alleles were not genetically associated with major genes for callus colour. Thus genotype determination at these loci when considered together with pigmentation characters may be valuable taxonomically for further distinguishing between the species.

Introduction

The two Australian species of fruit fly *Dacus* (*Strumeta*) *tryoni* and *Dacus* (*Strumeta*) *neohumeralis* Birch (Birch 1961) provide a striking example of gene pool isolation accompanied by only very little morphological differentiation and, in areas where they are sympatric, by little apparent differentiation in their ecology. The broad ranging *D. tryoni* is found along the eastern coast of Australia extending from northern Queensland to northern Victoria whereas *D. neohumeralis* has a more restricted distribution along only the north-eastern coast being found no further south than Coffs Harbour, about 200 miles north of Sydney.

These two species hybridize in the laboratory (Bateman 1958; Gibbs 1968). In 1966 Lewontin and Birch hypothesized that they also hybridize in the field, at least to some small extent, and that this gene exchange provided in one species a greater diversity of genes on which selection could act. This, they suggested, enabled *D. tryoni* to rapidly adapt to new environments and so to spread southwards as seems to have been happening with this species over the last 100 years. The feasibility of an event such as this has been demonstrated under laboratory conditions by Lewontin and Birch.

The question of field hybridization, however, still rémains—is there introgression in the field that would cause an increase in diversity of genes? The difficulty in answering this question is that the taxonomic character used for distinguishing the two species (the extent of yellow or brown pigmentation on the humeral calli, see Fig. 1) forms a continuum in populations of the two species. Even using five different pigmentation characters there is still a small proportion of individuals which cannot be assigned to one or other species (Vogt and McPherson 1972). These individuals may be either hybrids or members of one or other species. If hybridization occurs in contemporary populations, then it possibly is a causal factor in the maintenance of this observed taxonomic variation (see Birch and Vogt 1970).

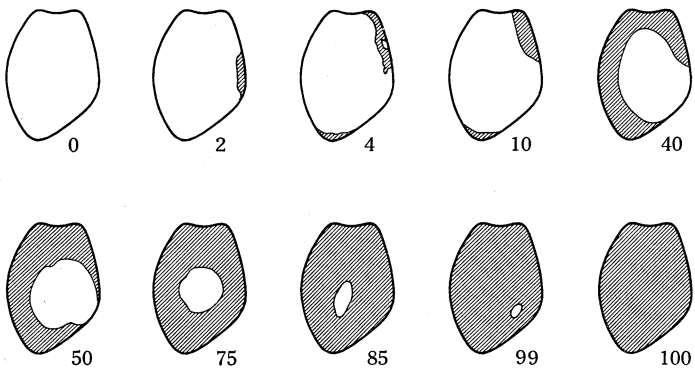

Fig. 1. The range of area of brown pigment on the humeral callus showing the upper limits for each callus category. The number beneath each diagram is the estimated mean percentage of brown pigment on the callus of each category (from Birch and Vogt 1970).

This study was aimed at finding a means of identifying individuals as members of one or other species or as hybrids. Initially a survey of 15 specific proteins was carried out by electrophoresis using established laboratory populations of both species (McKechnie 1972). Only three of these proteins proved to be polymorphic and of any interest with respect to species differentiation. The remaining proteins were largely monomorphic and identical in both species. In this paper gene–enzyme variation in natural populations is described for these three interesting proteins, an alcohol dehydrogenase (Adh), an octanol dehydrogenase (Odh) and an esterase (E-2).

Materials and Methods

Flies were sampled from four localities during the summer of 1970/71: Wollongong and Sydney in the south where *D. tryoni* occurs in the absence of *D. neohumeralis*, and Brisbane and Rockhampton in the north where both species abound. Fruit infested with larvae were collected and electrophoresis was carried out on the adults which subsequently emerged in the laboratory. Routine rearing techniques have been described by Birch (1961) and by Bateman (1967). Gene frequency estimates for *D. tryoni* from Wollongong and Sydney were based on random samples of flies which emerged from the fruit. In these samples flies were not sorted on the basis of callus colour, though all were inspected and no fly with a callus of more than 80% brown occurred. For the northern samples, flies were sorted into species groups on the basis of callus colour. Flies with an all brown (100% brown) callus were taken for the *D. neohumeralis* sample, and flies with a callus of 0–3% brown were taken for *D. tryoni*. Electrophoresis was not carried out on the few callus

colour intermediates obtained in these collections. Methods of electrophoresis, the inheritance of the three polymorphic loci studied and a demonstration of likely homology of the alcohol dehydrogenase locus in both species, have been described (McKechnie 1974). On every gel at least one standard reference protein was included for comparative purposes.

In the field samples some 'new' alleles at the E-2 locus were found, being alleles which had not been previously detected in laboratory cultures. If there was any uncertainty about the relative mobility of these proteins they were put into allele groups, each of which contained only alleles with very similar electrophoretic mobility. The most common 'new' allele to turn up in field samples was a null, or silent, allele, $E\text{-}2^{null}$. The strongest evidence for this was among Brisbane *D. tryoni* where several individuals failed to display any E-2 enzyme activity. Also, for both species among the northern samples there were significant E-2 heterozygote deficiencies. Very marked improvements of fit to Hardy–Weinberg equilibrium were obtained (Table 1) when null allele frequencies were estimated by the method of Zouros and Krimbas (1969). At the E-2 locus the estimates of frequencies of null alleles have been made for those field samples where significant heterozygote deficiencies occurred.

Table 1. Demonstration of the presence of a null allele by comparison, in two consecutive generations, of observed and expected genotype frequencies at the E-2 locus in *D. tryoni* from Brisbane

Null allele is assumed both absent (A) and present (P)[A]

		Phenotype							Total	χ^2	d.f.	Gene frequencies			
		M	M/0·95	M/N	0·95	0·95/N	N	—/—				M	0·95	N	Null
G_0[B]	Obs.	5	11	23	17	12	30	1	99	—					
	Exp. (A)	4·9	12·8	21·3	8·3	27·7	23·0	0	98[C]	20·5***	3	0·22	0·29	0·48	—
	Exp. (P)	10·9	10·1	18·4	11·1	18·8	28·0	1·7	99	10·09**	2	0·22	0·23	0·42	0·13
G_1	Obs.	16	23	14	19	21	24	2	119	—					
	Exp. (A)	10·5	24·6	24·6	14·3	28·7	14·3	0	117[C]	20·22***	3	0·30	0·35	0·35	—
	Exp. (P)	16·7	19·0	17·1	22·8	21·2	19·6	2·6	119	3·25	3	0·25	0·31	0·29	0·15

** $P < 0·01$. *** $P < 0·001$.

[A] To calculate expected Hardy–Weinberg frequencies the locus was reduced to one with three active (non-null) 'alleles', by pooling lower frequency alleles with closely migrating alleles at higher frequencies. Group M: 1·08, 1·00, 0·98; Group N: 0·91, 0·86, 0·80.

[B] These adults were collected as larvae in the field and enclosed in a laboratory cage.

[C] Individuals which displayed no E-2 bands were omitted from sample.

To demonstrate independence of genes for callus colour and some of the allozymes found associated with these colour groups, selection for callus colour was carried out on four lines, duplicate lines being used for each species. From a *D. neohumeralis* laboratory population (see McKechnie 1974) four lines were derived. Two of these were unselected control lines and two were selected for a *tryoni*-like callus. In each generation, in each of the two selected lines, about 3000 flies were examined for callus colour (3–6 days after eclosion) and of these from 150 to 250 were selected and mated in mass for the next generation. For the control lines, 250 adults were selected at random and transferred. In each generation before selection 100 flies (50 of each sex) were scored for callus colour as described by Vogt (1970). A similar program was carried out on *D. tryoni* from an established laboratory population (origin: Sydney) where selection in the duplicate lines was for a *neohumeralis*-like callus.

Results

There was a marked similarity of gene frequencies within callus groups for all three loci over the entire geographic range, a distance for *D. tryoni* of approximately 1200 miles (see Table 2). This is not too surprising in view of recorded migration distances (up to 16 miles) and known movement patterns for *D. tryoni* (Fletcher 1974).

The Adh locus is by far the most useful for distinguishing the species. At this locus gene frequencies in field samples are similar to those found previously in laboratory populations (see McKechnie 1974). Both samples of *D. neohumeralis*

(brown-callus flies) were polymorphic with two alleles present, the major one, $Adh^{1\cdot20}$ occurring at a frequency of about 0·85. This allele was not detected in *D. tryoni* (yellow-callus flies) which themselves were monomorphic in all populations for the minor allele $Adh^{1\cdot00}$ (which occurred at low frequency in *D. neohumeralis*).

Table 2. Estimated gene frequencies at the Adh, E-2 and Odh loci in *D. tryoni* and *D. neohumeralis* from four localities

Locus and alleles	*D. tryoni*				*D. neohumeralis*	
	Rockhampton	Brisbane	Sydney	Wollongong	Rockhampton	Brisbane
Adh: 1·00	1·00	1·00	1·00	1·00	0·14	0·15
1·20	—	—	—	—	0·86	0·85
Sample size	60	68	80	80	60	44
E-2: 1·08(A)[A]	—	0·01	—	—	0·03	0·01
1·00	0·34	0·20	0·43	0·34	0·46	0·40
0·98	0·06	0·01	0·07	0·07	0·24	0·18
0·95(B)[A]	0·13	0·23	0·12	0·28	0·11	0·17
0·91 (C)[A]	0·38	0·40	0·38	0·23		
0·86 (C)[A]	—	0·01	—	0·05	0·02	0·04
0·80 (C)[A]	—	0·01	—	—		
Null	0·09	0·13	—	0·03	0·14	0·20
Sample size	99	99	95	129	111	59
Odh: 1·00	0·95	0·96	0·94	0·90	1·00	1·00
0·88	0·05	0·04	0·06	0·10	—	—
Sample size	60	68	80	80	60	44

[A] A, B and C groups of alleles for the *D. neohumeralis* samples. The alleles in a group migrate to a similar distance and their pooled total frequencies are given. Groups A, B and C may include the 1·08, 0·95 and 0·91, 0·86 and 0·80 alleles, respectively, of *D. tryoni*.

Allele frequencies at the Adh locus obviously show a strong association with callus colour. If this association results from tight linkage of the Adh locus and major genes which determine callus colour, or from pleiotropic effects, then we would simply have two views of a single taxonomic character. To demonstrate independence of these two characters, duplicate populations of brown-callus flies were selected towards a yellow callus for four generations and gene frequencies at the Adh locus compared to duplicate unselected control lines. Wolda (1967) had shown that callus colour can easily be selected and that four generations is sufficient to completely change callus colour distributions from that of one species to that of the other. Although brown callus colour genes were virtually eliminated from the selected lines (Fig. 2) there was no marked decrease in frequency of the $Adh^{1\cdot20}$ allele (Table 3). Gene frequencies at the Adh locus, then, represent a difference between *D. tryoni* and *D. neohumeralis* gene pools and not just a difference between callus colour groups. The implication is that Adh genes and major genes for callus colour are segregating independently in natural populations and that by taking a sample of 100% brown-callus flies we have obtained a real estimate of Adh gene frequencies for *D. neohumeralis*.

At the esterase locus, E-2, there were two notable consistent differences between callus colour groups in these samples. First, in *D. neohumeralis* the 0·98 allele occurred at frequencies of 0·24 and 0·18, significantly greater than its highest frequency of 0·07 among *D. tryoni* samples. Second, in *D. neohumeralis* alleles

with a relative mobility around 0·86 (see Group C, Table 2) were fairly rare at frequencies of 0·02 and 0·04. On the other hand in *D. tryoni* these alleles occurred at higher frequencies; frequencies ranging from 0·28 to 0·42. Clearly, at this locus there also is an association of allele frequencies with callus colour.

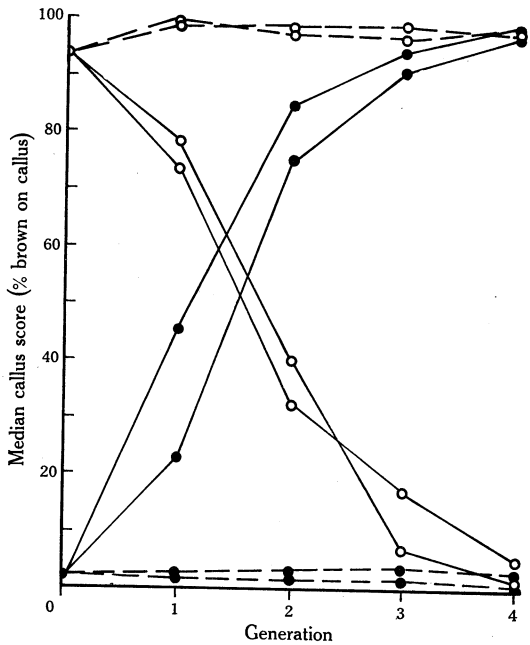

Fig. 2. Response to selection for callus colour in *D. tryoni* (●) and *D. neohumeralis* (○). *D. tryoni* was selected towards an all brown (*neohumeralis*-like) callus and *D. neohumeralis* was selected towards an all yellow (*tryoni*-like) callus; both in duplicate. Duplicate unselected control lines were included for each species. ——— Selected line; – – – control line.

Table 3. Comparison of gene frequencies in duplicate selected lines with duplicate control (unselected) lines for (a) the Adh locus in *D. neohumeralis* (selected for yellow callus) and (b) the E-2 locus in *D. tryoni* (selected for brown callus)

Alleles	Control line 1	Control line 2	Selected line 1	Selected line 2
(a) *D. neohumeralis*, Adh locus				
1·00	0·19	0·17	—	0·24
1·20	0·81	0·83	1·00	0·76
Sample size	40	39	43	36
(b) *D. tryoni*, E-2 locus				
1·00	0·69	0·60	0·54	0·70
0·95	0·10	0·11	0·01	—
0·91	0·21	0·29	0·45	0·30
Sample size	78	60	100	79

To demonstrate independence of the E-2 locus and genes for callus colour similar duplicate selection lines were established. This time *D. tryoni* was selected towards a brown callus and gene frequencies at the E-2 locus were compared with those of unselected lines (see Table 3). Again, though yellow-callus genes were

eliminated (Fig. 2) there was no decrease in frequency of the 0·91 allele which has been shown above to be associated in natural populations with yellow callus. Thus, it seems likely that at least this allele at the E-2 locus segregates, in natural populations, independently of genes for callus colour.

At the octanol dehydrogenase locus both samples of *D. neohumeralis* were entirely monomorphic for one allele, $Odh^{1\cdot00}$. On the other hand, all *D. tryoni* samples were dimorphic with a second allele, $Odh^{0\cdot88}$, occurring at relatively low frequency, frequencies ranging from 0·04 to 0·10 (Table 2). It seems unlikely that the $Odh^{0\cdot88}$ allele would be closely linked to major genes responsible for yellow callus, since it was not found in a large proportion of yellow-callus flies.

Discussion

Though the sample sizes in this study have not been large, some insight into the taxonomic value of these three loci can be gleaned. None of the three loci considered alone can be used to discriminate between species. In the populations studied the Adh locus comes closest to being a good species discriminator. In *D. neohumeralis* 97% of individuals had the $Adh^{1\cdot20}$ allele in their genome. This allele was not detected in the other 3% nor in any of the 288 *D. tryoni* sampled—all of which were homozygous for the $Adh^{1\cdot00}$ allele. Thus there is no indication of any introgression of the $Adh^{1\cdot20}$ gene into the *D. tryoni* gene pool. Both the E-2 and Odh loci also show species specific allelic distributions. These results strongly support the findings of others (Birch 1961; Wolda 1967; Vogt 1970) that we are dealing with two distinct gene pools.

By considering a fly's genotype at all three loci, improved species separation may be obtained. Clearly, if all of the five pigmentation characters described by Birch and Vogt (1970) were considered together with these protein genotypes, virtually complete division of the two species groups would result. In light of the species-discerning ability of allozymic variation in *Drosophilla* (Ayala and Powell 1972), another, perhaps more profitable, approach to this problem would be to look at many new enzymes which were not among the fifteen surveyed by McKechnie (1972) and for which assay methods are available (Shaw and Prasad 1970; Ayala *et al.* 1972).

Evidence of contemporary hybridization might be obtained by a comparison of the frequency of the $E\text{-}2^{0\cdot91}$ allele in a sample of flies which are both callus intermediates and Adh heterozygotes, with the frequency of this allele in *D. neohumeralis*. Since this allele is associated in nature with yellow callus an increase in its frequency among Adh heterozygotes with an intermediate callus would strongly indicate gene exchange. Unfortunately, since the frequency of $E\text{-}2^{0\cdot91}$ is not large and since we know that hybridization occurs at a very low frequency, if it occurs at all, this method would require a large sample that would be very difficult to obtain as well as to analyse.

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