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Geographical polymorphism of amylase in *Drosophila ananassae* and its relatives

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Strains of *Drosophila ananassae* from various places in the Tropics were investigated for their electrophoretical amylase pattern. Eleven isoamylases were found in adult flies. African populations were much more polymorphic than those from the Far East, and showed multibanded phenotypes, suggesting a multiplication of the *Amy* structural gene, with at least four copies per haploid genome in certain populations. Nine other species of the *D. ananassae* subgroup had weak amylase activity and only one or two variants were found in each species. *D. monieri* and *D. varians* are closely related to *D. ananassae* and showed a single band, similar to the isoamylase 3 of *D. ananassae*, which suggests that this might be an ancestral allele.

INTRODUCTION

Amylase has been widely studied in *Drosophila*. Following initial genetic experiments carried out by Abe (1958) and Kikkawa (1960), Kikkawa (1964) and Doane (1964) described several electrophoretic variants of this protein in *D. melanogaster*. Daïnou *et al* (1987) reported 12 different isoamylases in this species, which is highly polymorphic in Africa (Hickey, 1979; Daïnou, 1985) and less variable in other parts of the world. *D. melanogaster* was more variable than the other species of the *D. melanogaster* subgroup (Daïnou *et al*, 1987). The *Amy* locus is duplicated in *D. melanogaster* (Bahn, 1967) and throughout the *D. melanogaster* subgroup (Daïnou *et al*, 1987; Payant *et al*, 1988). As the amylase duplication has also been found in the *D. obscura* group (Doane and Norman, 1985; Cariou, unpublished data), it is interesting to know whether this extends to other species within the *D. melanogaster* group.

The *D. ananassae* subgroup is included in the *D. melanogaster* group and is divided into three complexes: *ananassae*, *bipectinata* and *ercepeae*. All the 21 presently known species are tropical. Two of them are circumtropical, *D. ananassae* and *D. malerkotliana* (David and Tsacas, 1981), and most of the others are endemic to Pacific archipelagos or to South-East Asia where *D. ananassae* is thought to be native (Dobzhansky and Dreyfus, 1943; McEvey *et al*, 1987). A few species have been found in the Afrotropical region: *D. lachaisei*, *D. parabipectinata*, *D. ercepeae*, *D. vallismaia* (Tsacas, 1984; Lemeunier *et al*, 1986). *D. ananassae* is a domestic species, sometimes abundant in human habitats. It exhibits some unusual genetic features, such as spontaneous male crossing over, segregation distortions, very high inversion frequency, translocations and high mutability (Singh, 1985).

Here we analyse the amylase electrophoretic patterns in numerous strains collected around the Tropical belt.

MATERIALS AND METHODS

The characteristics of the populations or strains of *D. ananassae* studied are given in table 1. Some of them have been recently collected while others have been kept in the laboratory since the early sixties.

The related species studied are the following: *D. malerkotliana* (one mass culture from Ivory Coast, three isofemale lines from Madagascar and eight isofemale lines from Ecuador); *D. bipectinata* (strain, New Caledonia); *D. parabipectinata* (17 isofemale lines, Mauritius); *D. pseudoananassae* (Thailand, the *nigrens* strain from the Bowling Green Stock Center); *D. ercepeae*-like (strain, Madagascar); *D. ercepeae* (strain, Réunion

Table 1 Strains of *D. ananassae* used in this study. For each isofemale line, at least three individuals were tested.

Name	Geographical origin	Symbol	Type of culture	Date of collection	Number of individuals
14024-0371-4	Samoa	Sa	Lab. strain*	1965	24
14024-0371-8	Palmyra	Py	Lab. strain*	1962	9
14024-0371-13	Tonga	Tg	Lab. strain*	1962	9
14024-0371-3	Hawaii	Hw	Lab. strain*	1962	7
14024-0371-15	Palau	P	Lab. strain*	1965	7
Takapoto	Tuamotu	Tk	16 isofemale lines	1986	253
Moorea	Societe Is.	M	8 isofemale lines	1986	46
Mexico	Mexico	Mx	2 isofemale lines	1987	45
São Paulo	Brazil	SP	1 isofemale line	1987	9
Guadeloupe	West Indies	G	Mass culture	1986	22
			5 isofemale lines	1985	22
Bouaké	Ivory Coast	B	3 isofemale lines	1987	16
Taï	Ivory Coast	T	Mass culture	1983	74
Cotonou	Benin	C	3 isofemale lines	1987	33
Djeffa	Benin	Dj	8 isofemale lines	1987	134
			Wild flies	1987	134
Brazzaville	Congo	Bz	Mass culture	1987	17
Maroantsetra	Madagascar	Mt	19 isofemale lines	1987	106
Andasibe	Madagascar	A	3 isofemale lines	1987	24
Réunion	Réunion Is.	R	Mass culture	1987	15
Varanasi	India	V	7 isofemale lines	1987	44
Noumea	New Caledonia	N	Mass culture	1987	24

* Bowling Green *Drosophila* Stock Center

Island); *D. vallismaia* (strain, Praslin, Seychelles); *D. monieri* (mass culture, Moorea); *D. varians* (Philippines, strain from the Bowling Green Stock Center).

Flies were reared on cornmeal standard medium and fed as adults with killed yeast medium for at least 24 h prior to electrophoresis. This medium does not contain sugars, which, in *D. melanogaster*, repress amylase synthesis (Benkel and Hickey, 1986). Single flies were homogeneized in 15 μl distilled water, then 30 μl of a 40 per cent saccharose solution was added. Samples were electrophorized on a 5 per cent polyacrylamid gel in a buffer made of Tris 0·1 M, borate 0·05 M, *p*H 8·9. After running, gels were incubated for $1\frac{1}{2}$ hours in a 1 per cent starch solution containing $CaCl_2$ 25 mM, Tris 25 mM, *p*H 7·5, then stained using Lugol.

RESULTS

Polymorphism in Drosophila ananassae

Eleven different isoamylases were identified in *D. ananassae* adults. They are named either by convenient single numbers (as in *D. melanogaster*) or with respect to their relative mobilities: the isoamylase "3" corresponds to a mobility of 100 (figs 1 and 2).

The Afrotropical region shows much higher polymorphism than elsewhere, in addition few isoamylases are found in the Australasian region (fig. 3). The two isoamylases, *Amy* 3 and 4 are present throughout the Tropical region. Two other isoamylases, *Amy* 2 and *Amy* 2′ are very close to each other and are not easy to separate, particularly in heterozygotes. The frequencies of *Amy* 2 and *Amy* 2′ were pooled when both were present. Two isoamylases are common in various countries: *Amy* 1 and *Amy* 2′ (table 2). Populations from Africa and Madagascar show a large variety of phenotypes while the Polynesian flies have only three, corresponding to the 2 isoamylases present. Single-banded phenotypes are very abundant in the Pacific islands and very rare in Africa, where unusual complex patterns with more than two bands (up to 5) are predominant. Since no *amy-null* mutant has been found in *D. ananassae*, we have not been able to study haplotypic structure. The frequencies of the different isoamylases are given in table 3. *Amy* 3 and 4 are the most common; they are almost fixed, but in some populations *Amy* 4 is absent (Palmyra, Tonga, Palau) and in others some individuals lack the *Amy* 3 band. For example, a strain homozygous for isoamylase 4 has been derived from the Takapoto population. *Amy* −1 is rare in the wild but a high frequency strain has been selected from the Taï population.

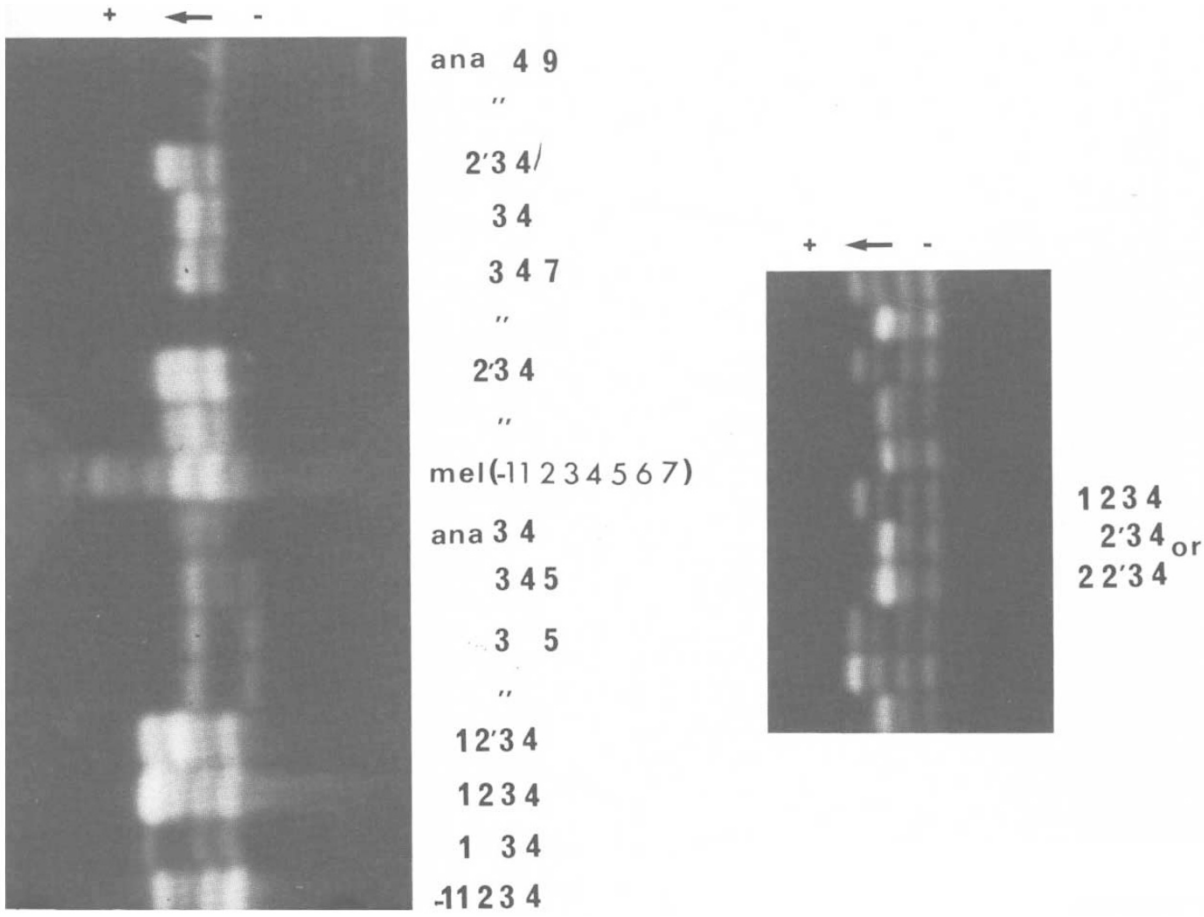

Figure 1 Various phenotypes of *D. ananassae* individuals, showing most of the isoamylases identified in the species. Complex phenotypes with five bands are clearly visible. Amylase variants of *D. ananassae* may be compared on this gel to the position of the *D. melanogaster* isoamylases.

Table 2 Percentages of different phenotypes in a few African and Pacific populations. *Amy* 9 has not been considered here (Polynesian populations). *Amy* 2* means that *Amy* 2 and/or *Amy* 2' are present (Djeffa population)

Strain	Djeffa mass	Djeffa isofemale	Maroantsetra	Moorea	Takapoto
Phenotype					
Amy 3	0·5	0	6	87	56·5
Amy 4	0	0	1	0	3·5
Amy 3, 4	30·5	30	56	13	40
Amy 2*, 3	1	0	—	0	0
Amy 2', 3	—	—	3	0	0
Amy 2*, 4	1	3	0	0	0
Amy 2*, 3, 4	55	45	—	0	0
Amy 2', 3, 4	—	—	27	0	0
Amy 1, 3, 4	4	8	0	0	0
Amy 1,2*, 3, 4	4	1	0	0	0
Amy 3, 4, 5	0	0	4·5	0	0
Amy 2', 3, 4, 5	0	0	2	0	0
Amy 3, 4, 7	1	4	0	0	0
Amy 2*, 3, 4, 7	0·5	7	0	0	0

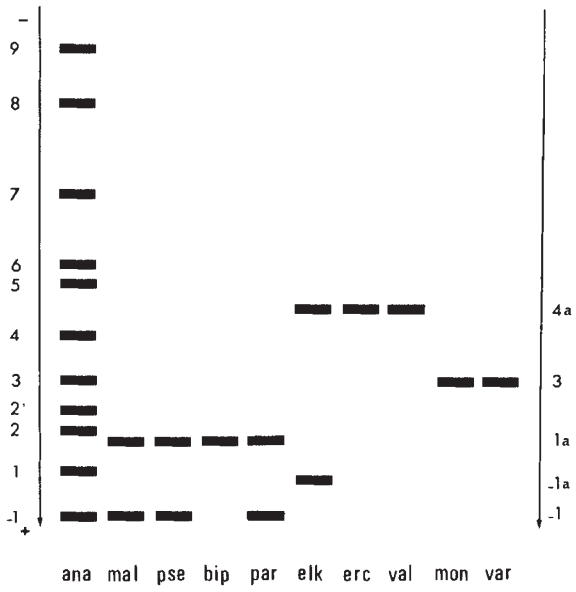

Figure 2 Relative positions of the different isoamylases found in *D. ananassae* (ana); *D. malerkotliana* (mal); *D. pseudoananassae* (pse); *D. bipectinata* (bip); *D. parabipectinata* (par); *D. ercepeae*-like (elk); *D. ercepeae* (erc); *D. vallismaia* (val); *D. monieri* (mon); *D. varians* (var). Position 100 was given to the most common allele of *D. ananassae* (*Amy* 3).

Amy 1 and 2′ are common in Africa, the Indian Ocean and Tropical America, with a high frequency for *Amy* 2′ in the Equatorial populations of Africa. *Amy* 2 is reported from Equatorial Africa. The other isoamylases are geographically limited: *Amy* 5 to Madagascar and Guadeloupe, *Amy* 6 (observed only once) and 7 to Benin and *Amy* 9 to French Polynesia. A very slow band similar to *Amy* 9 has been reported in species of the *D. melanogaster* subgroup (*D. melanogaster*, *D. simulans*, *D. teissieri*) where it has not been ascribed with certainty to the usual gene-enzyme system (Cariou, unpublished data). *Amy* 8 appears in a few individuals from Africa.

The amylase pattern in other species of the D. ananassae *subgroup*

Fig. 2 shows that for all the species studied here, single banded phenotypes are predominant. *D. malerkotliana*, *D. bipectinata*, *D. parabipectinata*, and *D. pseudoananassae* have a common band, slightly faster than the *Amy* 2 of *D. ananassae*. That band may correspond to the same allele for these four species. In addition, two isofemale lines of *D. malerkotliana* (one from Madagascar and one from Ecuador), and some individuals of *D.*

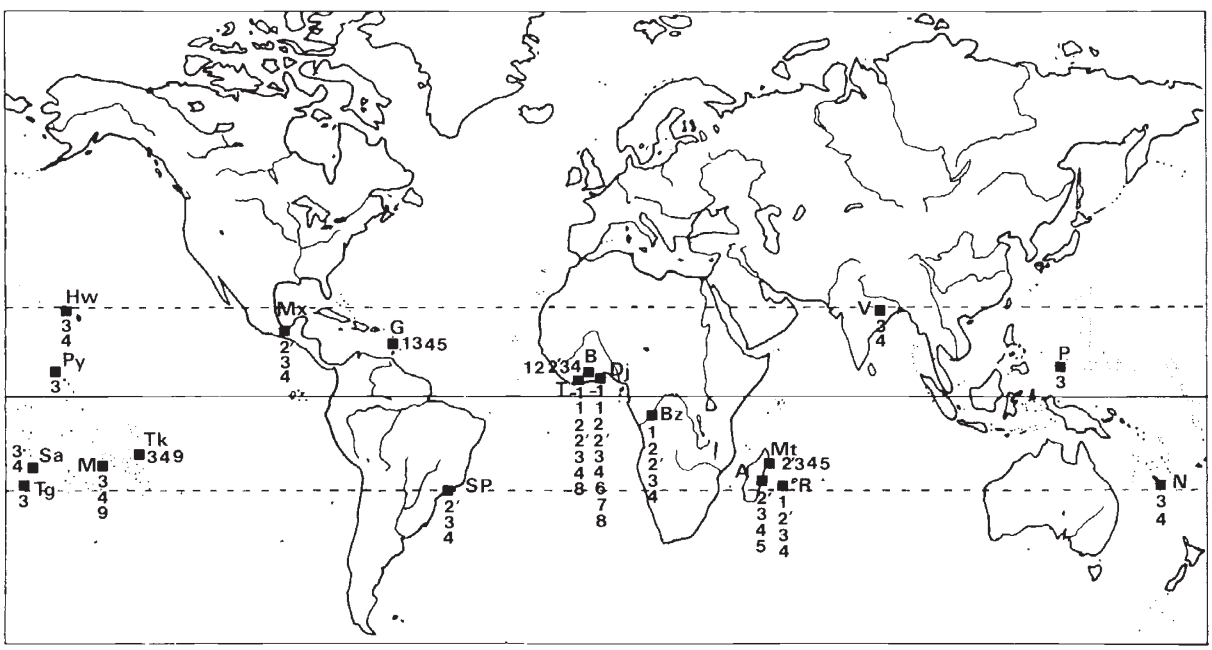

Figure 3 Geographical distribution of amylase isozymes in *D. ananassae*. Symbols of localities are given in table 1.

Table 3 Percentages of individuals carrying each isoamylase in the different localities

Isoamylases	−1	1	2	2′	3	4	5	6	7	8	9
Strains											
Samoa	0	0	0	0	100	80	0	0	0	0	0
Palmyra	0	0	0	0	100	0	0	0	0	0	0
Hawaii	0	0	0	0	100	100	0	0	0	0	0
Tonga	0	0	0	0	100	0	0	0	0	0	0
Palau	0	0	0	0	100	0	0	0	0	0	0
Takapoto	0	0	0	0	96·5	43·5	0	0	0	0	see text
Moorea	0	0	0	0	100	20	0	0	0	0	see text
Mexico	0	0	0	29	100	96	0	0	0	0	0
São Paulo	0	0	0	22	100	100	0	0	0	0	0
Guadeloupe	0	11	0	0	100	1001	8	0	0	0	0
Bouaké	0	25	28		100	100	0	0	0	0	0
Taï	exists	41	35		99	100	0	0	0	exists	0
Cotonou	5	19	76		100	100	0	0	0	0	0
Djeffa mass	exists	7·5	64		99	98	0	0·7	1·5	1·5	0
Djeffa isofemale	0	10·5	56		97	100	0	0	11	0	0
Brazzaville	0	8	80		100	100	0	0	0	0	0
Maroantsetra	0	0	0	32	99	91	6·5	0	0	0	0
Andasibe	0	0	0	8	100	96	8	0	0	0	0
Réunion	0	33	0	33	100	100	0	0	0	0	0
Varanasi	0	0	0	0	100	100	0	0	0	0	0
Noumea	0	0	0	0	86	100	0	0	0	0	0

parabipectinata and *D. pseudoananassae* show a second, common band, faster than the former. *D. ercepeae*, *D. vallismaia* and *D. ercepeae*-like share a band which is slightly slower than *Amy* 4 of *D. ananassae*. *D. ercepeae*-like shows a second, faster isoamylase. Finally, *D. monieri* and *D. varians* express a single amylase band that has the same mobility as the *D. ananassae* isoamylase 3.

DISCUSSION

Many *D. ananassae* individuals, especially in Africa, show complex phenotypes with three, four and sometimes five bands. Because of the monomeric structure of the amylase protein, any electrophoretic pattern with more than two bands may indicate a multiplication of the coding gene. The duplication of the *Amy* locus is well established in the *D. melanogaster* subgroup (Boer and Hickey, 1986; Doane *et al*, 1987 Daïnou *et al*, 1987; Payant *et al*, 1988). In *D. ananassae* the exact number of gene copies remains questionable but more than two active copies must exist in at least some individuals or populations: the five-banded individuals strongly suggest a triplication of the *Amy* gene. A stable strain, homogeneous for *Amy* 2. 3. 4. and therefore considered to be homozygous for these three alleles, has also been derived from the Taï population. In addition, some crosses (data not shown) between an *Amy* 1. 2. 3. 4 male from Taï (where *Amy* 4 is fixed) and an *Amy* 4 female from Takapoto produce some *Amy* 1. 2. 3. 4 F1 individuals, indicating that not only *Amy* 1. 2 and 3, but also *Amy* 4, are transmitted paternally. Another cross with an *Amy* 3 female instead of *Amy* 4 leads to the same conclusion that four copies of the *Amy* gene may be present in the paternal haploid genome. We are not able yet to show whether each population of *D. ananassae* harbours the same number of copies. Some populations exhibit a single isoamylase, but a single-banding pattern is not evidence for a single locus (Doane *et al*, 1987).

D. ananassae also shows a marked geographic differentiation for amylase polymorphism: populations from Africa and the Indian Ocean are much more polymorphic and have the greatest allelic diversity. A similar geographic pattern has been reported in *D. melanogaster*. For this species, Daïnou *et al.*, (1987) explained the high number of alleles in Africa using historical arguments, since *D. melanogaster* is thought to have originated in West Africa. In the case of *D. ananassae*, the ancestral populations probably lived in the Far East (Dobzhansky and Dreyfus, 1943). Populations from that region might then be expected to have high polymorphism. Our results do not support this, and the scarcity of alleles in South East Asia is difficult to explain. We lack data from

Indonesia, New Guinea and Australia and in some cases, we assayed very old laboratory strains probably founded by few individuals that might not be representative of natural populations.

D. ananassae and *D. melanogaster* are both domestic species which have spread from Africa to America through man's activities. Interestingly, African and American populations of *D. ananassae* share several alleles. The high polymorphism in Africa might suggest an ancient colonization of this region, but if the number of *Amy* gene copies is variable within the species, the difference of levels of polymorphism between Africa and Asia might also be explained by a higher number of functional loci in African populations.

The other species of the *D. ananassae* subgroup, which have weaker activity, show low polymorphism. Within the *D. ananassae* subgroup, our results are consistent with the taxonomy: *D. bipectinata*, *D. parabipectinata*, *D. malerkotliana*, and *D. pseudoananassae*, which constitute the *bipectinata* complex, and produce, in interspecific crosses, fertile F_1 females (Lemeunier *et al.*, 1986), show the same major isoamylase. *D. malerkotliana*, *D. parabipectinata and D. pseudoananassae* frequently share an additional band. *D. ercepeae*, *D. ercepeae*-like and *D. vallismaia* belong to the *ercepeae* complex and are more distantly related to *D. ananassae*. These three species show a common isoamylase. *D. monieri* and *D. varians* belong to the *ananassae* complex. Both have a single isoamylase similar to *Amy* 3 of *D. ananassae*. This result could suggest that *Amy* 3, the most widespread allele in *D. ananassae*, might be ancestral.

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