Interspecific ovarian transplantations in *Drosophila*: vitellogenin uptake as an index of evolutionary relatedness

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Evolutionary divergence is measured either by comparing homologous characters in related species or by assessing the compatibility of genes, cells or tissues from different species to sustain normal development and function. Here we examine vitellogenin accumulation in interspecifically transplanted ovaries as a measure of evolutionary divergence of the second kind. We report on the results from about 400 ovary transplantations involving 27 species from four different species groups of *Drosophila*. With the exception of one species, no vitellogenin accumulation was observed in inter-group transplantations, but accumulation occurred at varying degrees in intra-group transplantations. This degree is in good agreement with intra-group phylogenetic relationship established on other criteria and it can, therefore, be a useful complementary index of relative evolutionary divergence among members of the same species group.

INTRODUCTION

Most studies of evolutionary distance of related species have used characters whose degree of divergence is being studied without reference to (or in ignorance of) the character's "role" in the development or function of the organism. Chromosomal inversions (e.g., Wasserman, 1960), allozymes (e.g. Zouros, 1973), and variation scored at the DNA level (e.g., Coyne and Kreitman, 1986) are among the most typical examples of this type of character.

A second category of character that has also been used for the inference of evolutionary relatedness, relates to the degree to which combinations of genes, cells, or tissues from different organisms are compatible with the development of a normal phenotype. Because they are scored through the manifestation of their role, these characters cannot be treated as neutral to the forces of natural selection nor can they be assumed to have time-dependent rates of evolution. Also, they normally have two-state (yes-or-no) discontinuous distributions, in contrast to the continuous distributions of the characters of the first category. Given these differences, an important question is how concordant are the phylogenetic relationships derived from the two classes of character.

In *Drosophila*, the compatibility between genomes from different species has been studied mostly through hybridization which, however, can be applied only to very closely related species. The simple technique of transplanting immature ovaries from newly hatched females of one species (the donor species) into females of another species (the host species) is free of this limitation. In such transplantations the scored quantity is oocyte development in the guest (donor) ovary. Previous studies using this assay have largely concentrated on developmental aspects of vitellogenin production and uptake or on the general question of ovary growth in interspecific transplantations (Kambyzellis, 1970; Srdic *et al.*, 1978). Here we explore the possibility for the use of this assay as an index of phylogenetic relationship.

MATERIALS AND METHODS

The following *Drosophila* species were used. *Melanogaster* subgroup: *melanogaster*, *simulans*, *mauritanica*, *yakuba*, *erecta*, *teissieri*, *orena*; *montium* subgroup: *auraria*, *serrata*, *jumbulina*; *willistoni* group: *willistoni*, *tropicalis*, *pauistorum*, *equinoxialis*; *repleta* group: *mojavensis*,

arizonensis, *mulleri*, *hydei*, *eohydei*, *mercatorum*, *repleta*, *peninsularis*; *virilis* group: *virilis*, *americana*, *americana*, *montana*, *borealis*.

Transplantations of ovaries were performed as described before (King and Bodenstein, 1965; Postlethwait and Handler, 1978). Ovaries were removed from the donor species and transplanted into the host when both females were 2 hours old. Depending on the host species, host females were dissected 3 to 5 days after the operation, and the stage of oocyte development was scored in the guest ovary as well as in the two native ovaries. Transplanted ovaries were easily recognized from the native ones because they fail to attach to the host's oviducts. Oocyte development was measured using King's (1970) scale which recognizes fourteen developmental stages. Of these, stages 1 to 7 are pre-vitellogenic and 8 to 14 are vitellogenic. For each ovary the maximum developmental stage (MDS) reached by one or more oocytes was scored. For any donor/host combination we have performed several transplantations. For an overall measure of oocyte development for a given combination, we used the mean of MDS, as done previously by Kambyssellis (1970). We will refer to this mean as the index of maximum developmental stage (IMDS). The index is good only as an indication of general performance of transplanted ovaries, but it cannot be used for statistical inference. For comparing the results from transplantations involving two different donor/host combinations we used the Wilcoxon two-sample ranking non-parametric test (Brownlee, 1965).

An important condition of our experiments was that transplanted ovaries remained live within the host environment and did not lose their innate capacity for vitellogenin uptake if found in a permissible environment. The first condition was checked by observing, upon dissection of the host female, whether the transplanted ovary has grown at all. Atrophied, disintegrated or dead ovaries were not included in the data set. The second condition was checked by performing several "double transplantations" consisting in removing ovaries that have failed to develop to post-vitellogenic stages from their hosts (normally belonging to a different species group) and retransplanting them in females of their own species. Finally, to control for the possibility that a foreign ovary failed to develop not because of incompatibility with the host, but rather because of inability of the particular female host to sustain ovary development, we examined in each case the foreign as well as the two native ovaries.

RESULTS

Five types of "double transplantations" were performed: *melanogaster/virilis/melanogaster*, *serrata/melanogaster/serrata*, *willistoni/melanogaster/willistoni*, *virilis/hydei/virilis* and *equinoxialis/mercatorum/equinoxialis*. In each triad the first species was the original donor of the ovary, the second species was the first host and the third species was the second host. Each combination was repeated several times. In all cases the ovaries failed to develop while in the heterospecific host, but developed fully when transplanted in the conspecific host. These data illustrate the ability of ovaries to retain the potential for vitellogenin uptake when found in the appropriate environment, as was earlier observed (Srdic *et al.*, 1978).

The melanogaster species group

The taxonomy, biogeography, cytology, and cross-hybridization of this species group have been reviewed by Lemeunier *et al.* (1986). We have used 11 species from this group. Of these seven belonged to the *melanogaster* subgroup, three to the *montium* subgroup and one to the *ananassae* subgroup. Both species complexes (the *melanogaster* complex and the *yakuba* complex) of the *melanogaster* subgroup were represented among these species. The three *montium* species also belonged to different species complexes. The results are shown in table 1(a). The Wilcoxon two-sample rank test is significant for the *erecta/melanogaster* versus the *mauritiana/melanogaster* comparison ($u = 1.81$, $P = 0.035$) where the numbers of tested ovaries were relatively large, but it is not significant for other pair-wise comparisons (the next two most different comparisons *erecta/melanogaster* versus *melanogaster/melanogaster* and *erecta/melanogaster* versus *simulans/melanogaster* both yield $u = 1.58$, $P = 0.057$). The mean within-complex index (*simulans* or *mauritiana* ovaries transplanted into *melanogaster*) is different from the across-complex index (*yakuba*, *teissieri*, *orena* or *erecta* ovaries transplanted into *melanogaster*). The significance of the difference of the two means can be tested by pooling all within-complex scores into one group and all across-complex scores into another (this pooling is a permissible *a priori* operation given the well recognized subdivision of the *melanogaster* subgroup into two complexes). The Wilcoxon test for the two sets gives $u = 2.53$, $P = 0.006$. None of the three pair-wise comparisons in the *montium* subgroup are statistically different, nor is the intraspecific set

Table 1 Results from ovary transplantations among species of the *melanogaster* (a), the *willistoni* (b), the *repleta* (c) and the *virilis* (d) groups of *Drosophila*. The first species is the donor and the second is the host. *n* = number of successful transplantations, IMDS = the mean of maximum developmental stage, D = allozyme distance. Numbers in parenthesis are the maximum developmental stages of ovaries that produced no oocyte of stage 14. Taxonomic designations (in nested order) refer to the donor species. Results from statistical tests are given in the text

	<i>n</i>	IMDS	D
(a) <i>Melanogaster</i> group			
subgroup: <i>melanogaster</i>			
complex: <i>melanogaster</i>			
species: <i>melanogaster/melanogaster</i>	7	14	—
species: <i>simulans/melanogaster</i>	6	14	0.325
species: <i>mauritiana/melanogaster</i>	15	13.73 (10)	0.325
complex: <i>yakuba</i>			
species: <i>yakuba/melanogaster</i>	15	13.13 (10, 2X11, 12, 13)	1.099
species: <i>teisseiri/melanogaster</i>	6	13.00 (8)	0.811
species: <i>orena/melanogaster</i>	9	12.67 (9, 10, 11)	—
species: <i>erecta/melanogaster</i>	22	12.41 (3X8, 9, 10, 11, 2X12, 13)	1.099
subgroup: <i>montium</i>			
complex: <i>auraria</i>			
species: <i>auraria/auraria</i>	5	14	—
species: <i>auraria/melanogaster</i>	7	<7	—
complex: <i>serrata</i>			
species: <i>serrata/auraria</i>	13	12.31 (8, 9, 2X10, 11)	0.73
complex: <i>undefined</i>			
species: <i>jambulina/auraria</i>	8	13.75 (12)	—
subgroup: <i>ananassae</i>			
species: <i>ananassae/melanogaster</i>	6	<7	—
(b) <i>willistoni</i> group			
complex: <i>willistoni</i>			
species: <i>willistoni/willistoni</i>	4	14	—
species: <i>tropicalis/willistoni</i>	2	14	0.413
complex: <i>paulistorum</i>			
species: <i>paulistorum/willistoni</i>	13	10.53 (4X8, 3X9, 10, 12)	0.524
species: <i>equinoxialis/willistoni</i>	6	13.17 (10, 13)	0.656
(c) <i>repleta</i> group			
complex: <i>mulleri</i>			
species: <i>mojavensis/mojavensis</i>	12	14	—
species: <i>arizonensis/mojavensis</i>	13	14	0.212
species: <i>mulleri/mojavensis</i>	11	12.45 (2X8, 9)	0.324
complex: <i>hydei</i>			
species: <i>hydei/mojavensis</i>	23	12.74 (2X9, 3X10, 12)	—
species: <i>eohydei/mojavensis</i>	12	13.67 (10)	—
complex: <i>mercatorum</i>			
species: <i>mercatorum/mojavensis</i>	9	13.67 (11)	—
complex: <i>repleta</i>			
species: <i>repleta/mojavensis</i>	14	13.43 (9, 12, 13)	—
(d) <i>virilis</i> group			
phylad: <i>virilis</i>			
species: <i>virilis/virilis</i>	11	13.09 (2X9)	—
species: <i>americana amer./virilis</i>	15	11.60 (5X8, 9, 13)	0.540
phylad: <i>montana</i>			
species: <i>montana/virilis</i>	11	9.27 (5X8, 2X9, 3X10)	1.230
species: <i>borealis/virilis</i>	8	9.75 (8, 4X9, 2X10)	1.060

* D values for the *melanogaster* subgroup are from Eisses *et al.* (1979), for the *willistoni* group from Ayala *et al.* (1974), for the *repleta* group from Zouros (1973) and for the *virilis* group from Throckmorton (1982). The *serrata/auraria* distance was calculated from the data of Triantaphyllidis *et al.* (1978).

(*auraria/auraria*) different from the interspecific sets when they are pooled into one set. This is most likely the result of limited number of observations. The across sub-groups transplantations have produced quite different results. Two pairs of species were involved in these tests: *auraria/melanogaster* and *ananassae/melanogaster*. In both cases no vitellogenic oocytes were observed in any of the transplanted ovaries.

The willistoni species group

The evolutionary biology of the *willistoni* species group has been reviewed by Ayala (1975) who placed *willistoni* and *tropicalis* into one phylad, and *paulistorum* and *equinoxialis* (together with *pavlovskiana*) into another. This dichotomy is reflected in our data of ovary transplantations (table 1(b)). The Wilcoxon test for the *paulistorum/willistoni* versus *willistoni/willistoni* comparison is significant ($u = 1.98$, $P = 0.024$), and the comparison [*willistoni/willistoni* + *tropicalis/willistoni*] versus [*paulistorum/willistoni* + *equinoxialis/willistoni*] is also significant ($u = 1.69$, $P = 0.045$).

The repleta species group

Wasserman (1982) recognized 67 species in this group, and seven more remain to be described. The described species are assigned to five subgroups, mostly on the basis of cytological information. Our study involves seven species belonging to four subgroups. The results are shown in table 1(c). As it would be expected from the close phylogenetic relationship between *D. arizonensis* and *D. mojavensis*, there is no trace of inhibition of oocyte development in transplantations involving these two species. However, the third species in the complex, *D. mulleri*, produced a reduced index, but the difference cannot be established statistically (the comparison [*mojavensis/mojavensis* + *arizonensis/mojavensis*] versus *mulleri/mojavensis* gives $u = 1.29$, $P = 0.1$). In general, there is no significant heterogeneity in the scores from interspecific transplantations within the *repleta* group, and even the contrast [*mojavensis/mojavensis* + *arizonensis/mojavensis*] versus all other sets combined is barely significant ($u = 1.60$, $P = 0.055$).

The virilis species group

In his recent review of the *virilis* group Throckmorton (1982) lists 11 species assigned to two

“phylads”, the *virilis* and the *montana* phylad. We have examined four species of these groups, as shown in table 1(d). The intra-phylad contrast is not statistically significant (*virilis/virilis* versus *americana/virilis*, $u = 1.43$, $P = 0.076$), but both inter-phylad contrasts are significantly different (*virilis/virilis* versus *montana/virilis*, $u = 3.02$, $P = 0.001$; *virilis/virilis* versus *borealis/virilis*, $u = 2.44$, $P = 0.007$) and the [*virilis/virilis* + *americana/virilis*] versus [*montana/virilis* + *borealis/virilis*] contrast is also significant ($u = 4.46$, $P < 0.001$).

Inter-group transplantations

We performed 14 types of ovary transplantations in which donor and host species belonged to different groups. In six donor/host combinations both species belonged to the same subgenus (*Sophophora* or *Drosophila*) and in eight combinations the two species belonged to different subgenera. With two exceptions, oocytes failed to develop beyond the pre-vitellogenic stages. This result is not dependent on subgenus alliance (i.e., it does not matter whether the donor and host belong to the same or to a different subgenus of the genus *Drosophila*), and also is not host-dependent. The two exceptions involve the species *D. mercatorum*. Of seven *D. mercatorum* ovaries transplanted in *D. virilis*, one failed to develop beyond stage 7, two developed to stage 8, three to stage 12 and one to the final stage of 14. Similar results were obtained when the host species was *D. melanogaster*; of eight *D. mercatorum* ovaries, three failed to develop beyond stage 7, two developed to stage 13 and three to stage 14.

DISCUSSION

Mahowald and Kambyzellis (1980) have summarized the state of knowledge regarding vitellogenesis in *Drosophila*. In most species examined, there appear to be three major protein components in the egg yolk, all of which are synthesized in the fat body and are immediately secreted into the haemolymph. However, the ovary itself is capable of producing all main vitellogenin polypeptides as well as absorbing them from the haemolymph of its female host (Bownes, 1982). In our experiments the native ovaries of the host flies developed normally, which means that the presence of a foreign ovary did not interfere with the initiation and continuation of vitellogenin production or with its

assembly by the native oocytes. Thus, the failure of foreign oocytes to develop must be attributed to interspecific incompatibilities interfering with the uptake process.

Our purpose was not to study the basis of these incompatibilities, but rather to examine if there was a relation between the degree of the incompatibility and the phylogenetic distance between donor and host species. The outcomes from our transplantations fall into two discrete types. (1) Complete inability of foreign oocytes to incorporate host vitellogenin (no oocyte above stage 7), and (2) ability to incorporate vitellogenin (all ovaries with MDS above stage 7).

Intergroup transplantations belong to the first category. In this category also belong transplantations between subgroups of the *melanogaster* species group. In this respect, these subgroups behave as belonging to different species groups. Indeed, independent evidence suggests that the evolutionary distance between the *melanogaster* subgroups is much larger than the distance between the phylads of the *virilis* group or between the subgroups of the *repleta* group. This can be seen, most easily, in the study of Ohnishi *et al.* (1983) who have produced genetic distances between species belonging to *melanogaster* and *virilis* groups from two-dimension gel electrophoresis of total body proteins. Distances between species of the *melanogaster* complex or between species of the *montium* subgroup were about 0.20, distances between species belonging to different complexes of the *melanogaster* subgroup or to different phylads of the *virilis* group were about 0.35, but the distance between the *melanogaster* and *montium* subgroups was about 0.80. The opposite type of observation pertains to the *repleta* subgroups. Here the between subgroups values of IMDS are as high as the values between complexes of the *melanogaster*, *montium* or *willistoni* groups.

Intragroup transplantations belong to the second type, the one where vitellogenin accumulation in the donor ovary is possible. In this category IMDS varies among donor/host combinations. The most useful observation of our study is that this variation is in broad agreement with accepted phylogenetic divisions within subgroups. This is true for the *melanogaster* subgroup where the index is higher for donors belonging to the *melanogaster* complex than for donors belonging to *yakuba* complex. It is also true for the *willistoni* and *virilis* groups where within-complex or within-phylad combinations give higher index values than inter-complex or inter-phylad combinations. It is not true, however, for the *repleta* group where the

index cannot differentiate between species subgroups.

To demonstrate further the correspondence between vitellogenin accumulation and phylogenetic relationship established from biochemical divergence, we have correlated IMDS with genetic distance from allozyme studies. We were able to obtain such distances for fourteen pair-wise combinations of species, as shown in table 1. The sources for the genetic distances are given in the footnote to the table. Several studies have reported allozyme distances between members of the *D. melanogaster* complex (Eisses *et al.*, 1979; Gonzalez *et al.*, 1982; Tsakas and Tsacas, 1984). The results from these studies are in general agreement. We have chosen to use the distances given by Eisses *et al.* (1979) because, in addition to species of the *melanogaster* complex, this study includes distances between species from the *melanogaster* and *yakuba* complexes. Electrophoretic distances from different studies are not strictly comparable because of variations in the technique and, mainly, because they may employ different sets of protein loci. Yet, as has been argued in other studies adopting a similar approach (*e.g.*, Coyne and Orr, in press), this lack of uniformity is expected to increase the noise around the correlation, rather than to introduce a systematic bias causing an artefactual correlation. The correlation is shown in fig. 1. Both, the parametric ($r = -0.60$, $P = 0.02$) and non-parametric (Kendall's $\tau = -0.43$, $P = 0.03$) correlation coefficients are significant (two tailed probabilities), thus reinforcing the conclusion that the ability to accumulate vitellogenin is negatively correlated with phylogenetic distance. (These tests are not strictly valid because the genetic distances of species belonging in a cluster from a species outside the cluster are the same [Felsenstein, 1985]. Taking this into account the parametric and non-parametric correlation coefficients with their probabilities become $r = -0.58$, $P = 0.03$; $\tau = -0.45$, $P = 0.01$).

D. mercatorum presents an exception to the rule that no vitellogenin accumulation occurs in inter-group transplantations. Ovaries of this species developed to vitellogenic stages not only in *D. mojavensis* (a species of the same group), but also in *D. virilis* and *D. melanogaster* (species of different groups). Srdic *et al.* (1978) have also noted that *D. mercatorum* ovaries developed in *D. virilis* and *D. melanogaster* as well as in *Zaprionus vittiger*, a species of a different genus. It is obvious from these results that interspecific ovary transplantations may occasionally produce results that

Figure 1 Plot of index of maximum developmental stage (IMDS) against allozyme distance (D) for 14 combinations of donor/host species. The species pairs and the actual values of IMDS and D are given in table 1.

defy evolutionary relationship. The frequency and the degree of this defiance, as well as its causes, remain to be determined.

In this study we have attempted to demonstrate that interspecific transplantations can be used as an auxiliary tool for the elucidation of the evolutionary relationship between closely related species of *Drosophila*. Previous studies (Kambysellis, 1970; Srdic, 1978) have addressed this question at a more general level and have illustrated a broad agreement between compatibility for vitellogenin uptake and evolutionary distance. Here we examine the possibility that the degree of uptake can be used for a finer clustering of related species. One firm conclusion is that the assay has no power beyond the species-group level. The index is the same (zero) whether one compares the *melanogaster* group with the *willistoni* group or with the *repleta* group, even though it is known that the former two groups are phylogenetically closer. Also, the assay cannot distinguish between cross-hybridizing species. The compatibility index between individuals from such species is as high as between individuals from the same species. But the assay appears to be useful for distinguishing between intermediate levels of divergence such as subspecies, species-complexes, or species-subgroups. These levels, together with cross-hybridizing complexes, present most difficulties in taxonomic studies, and also are intrinsically more interesting for the study of the dynamics of reproductive and morphological divergence.

Quite apart from the usefulness of the assay in taxonomic studies, the study has demonstrated that

the *functional compatibility* between sets of genes separated in the course of evolution into independently evolving units (species) is related to conventional genetic distance. Clearly, a much larger set of data, preferably including many more cases of reciprocal transplantations, will be needed to establish more firmly and measure more accurately the relationship between genetic distance and vitellogenin accumulation index implied by fig. 1. Normally, functional compatibilities (or developmental programs) can only be studied in very closely related species (as hybrid fertility, inviability or morphological abnormality). Assays that have the potential to measure these compatibilities in more distantly related species and express them in quantitative rather than qualitative terms are much in need. They alone could provide information for judging how well the rates of evolution of molecular characters, obeying a time clock [either because of neutrality (Kimura, 1983) or because of stochastic variation in selection coefficients (Gillespie, 1988)], are related to rates of divergence at loci that control various developmental processes. Our study suggests that the correlation may turn out to be good.

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