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Sexual isolation of genetically differentiated sympatric populations of *Drosophila melanogaster* in Brazzaville, Congo: the first step towards speciation?

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Two sympatric populations of *Drosophila melanogaster* were collected in the Brazzaville area in Congo, one from the suburban countryside and the other from a brewery located in the city. They were compared for several genetically determined traits including morphology, allozymes, microsatellites, cuticular hydrocarbons, and sexual behaviour. The two populations were similar to other African populations for morphological traits, but differed significantly from each other for all other characters. The countryside population resembled other African populations, whereas the urban population was consistently similar to European populations. Mating choice experiments showed incipient reproductive separation between the populations. In agreement with the hypothesis that *D. melanogaster* originated in Africa and spread to the rest of the world by invading human-modified habitats, we suggest that man-adapted fruit fly populations have returned 'back to Africa', and remained partially isolated from older native stocks.

Keywords: *Drosophila melanogaster*, microspatial variation, prezygotic isolation, sexual isolation, speciation.

Introduction

The partial reproductive isolation of populations from the same species is thought to be an initial step towards speciation. Experiments using laboratory strains have provided evidence of this, even though the development of this isolation in natural conditions is difficult to observe (Rice & Hostert, 1993). A review of many studies on *Drosophila* species shows that prezygotic isolation evolves faster than postzygotic isolation when levels of isolation are ranked against genetic distance (Coyne & Orr, 1997). This analysis also suggests that there is a greater prezygotic isolation between sympatric than between allopatric pairs of species. Hence, analysis of speciation should include a systematic study of traits involved in prezygotic isolation (Tregenza & Bridle, 1997), at least in complex animals such as *Drosophila*. The cosmopolitan species *Drosophila melanogaster* was, until recently, thought to be uniform over its whole world-wide range and to show no sexual isolation. Homogamy was never observed in multiple choice experiments using strains from natural populations from various parts of the world (Henderson & Lambert, 1982). The only observed differences were in the sexual activity of African and European populations (Cohet & David, 1980). However, incipient allopatric speciation was recently suggested by the finding of sexual isolation between a North American and an African (Zimbabwe) population (Wu *et al*., 1995; Hollocher *et al*., 1997a,b).

There may be a very different situation in the Brazzaville area (Congo), according to the genetic structure of sympatric populations of *D. melanogaster*, shown by allozymes (Vouidibio *et al*., 1989). The microspatial distribution of alcohol dehydrogenase (*ADH*) polymorphism is unusual, revealing two kinds of population. A genetically homogeneous group of field populations (hereafter called the ''countryside populations'') were collected in periurban areas on ripe fruit and rotting manioc residues. Another group of brewery populations (the 'urban populations') were collected

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from two breweries (Kronenbourg and Primus), and from bars in the city. Permanent populations living on beer residues were established in returned bottle stores, providing useful reference populations. These two groups of populations differed significantly in their ethanol tolerance and in the frequency of the *Adh-F* allozyme. Urban populations had an *Adh-F* frequency close to 80%, whereas that of the countryside flies was 3%. Moreover, the urban flies were more resistant to ethanol. The F_{ST} value between the two kinds of populations was also high (0.46). This value is much higher than between other pairs of African populations suggesting that there was reduced gene flow between them (Vouidibio *et al*., 1989). Finally, preliminary results strongly suggest that these two populations are sexually isolated (Paillette *et al*., 1993).

We have therefore extended our analysis of these two kinds of population in a study of several traits including morphology, allozyme and microsatellite frequencies, abdominal and thoracic pigmentation, cuticular hydrocarbons, courtship behaviour and sexual isolation. The results show limited gene flow and reproductive isolation. These findings have been used to develop an evolutionary scenario whereby a migrant population from Europe, or from a Europe-related strain, recently became established in this African city and did not mix with ancestral populations of the same species.

Materials and methods

Natural populations

Strains were collected in 1989 in Loua (countryside population) and in the Kronenbourg brewery (urban population). These two samples, also designated as 'L' and 'K', respectively, correspond to sites 9 and 7 in a previous study (Vouidibio *et al*., 1989). The distance between these sites is about 15 km. A population from Bordeaux (France) was used as a reference for European populations in mating choice experiments. Samples were kept in mass culture at 20°C on laboratory medium. The samples used in this work are similar to those described in 1986, 1987 and 1988 by Vouidibio *et al*. (1989) in their allozyme frequencies and ethanol tolerance (see Table 1).

Phenotypic and genetic differentiation

Morphological traits were measured as described in Capy *et al*. (1993), pigmentation of females as in David *et al*. (1985) for the thoracic trident, and as in Gibert *et al*. (1996) for abdominal pigmentation, and cuticular hydrocarbons were measured as in Antony *et al*. (1985). Data published by Chakir *et al*. (1996) were used for ethanol tolerance. Allozyme frequencies were estimated at four loci in addition to *Adh*: *αGpdh* (α-glycerophosphate dehydrogenase), *Est-6* (esterase-6), *Est-C* (esterase-C) and *G6pd* (glucose-6-phosphate dehydrogenase) as in David (1982).

Microsatellite frequencies were estimated from trinucleotide repeats, as in earlier work conducted on European and African populations (Michalakis & Veuille, 1996). In the present study, data from France (sum of Bordeaux and Cognac), Ivory Coast (Lamto) and Malawi (see appendix B of Michalakis & Veuille, 1996) were compared to those of the countryside and urban populations of Brazzaville. These repeats are located in coding region of genes involved in development, which may be less subject to local adaptation than allozymes involved in interactions between the organism and its environment. We used six microsatellite loci (*bib*, *cad*, *dL*, *Elf-1*, *slobo*, *Su*(*H*)), showing significant differences between Africa and Europe (Michalakis & Veuille, 1996). The population samples consisted of isochromosomal lines started from gametes directly obtained from wild-caught individuals. We used a sample of 10 chromosomes of the Brazzaville populations. These may have undergone a significant loss of variation.

The characteristics of the two Brazzaville populations were compared to data published for other African and temperate populations (see Table 1).

Mating choice experiments

Three series of experiments were carried out. The first involved three individuals in all possible combinations; the second involved four individuals (one individual of each sex for each population); the third was a multiple choice experiment involving 50 individuals of each sex from each population (total number of individuals = 200). All experiments involved virgin males and females aged up to 5 days. Mating pairs are hereafter designated by indicating the strain of the female first, then the strain of the male. For example, an "LK" mating pair involved a female from Loua (L) and a male from Kronenbourg (K). Experiments with four individuals were also performed between Loua and Bordeaux (France) to compare the behaviour of allopatric and sympatric populations.

Because almost all the individuals from Loua were homozygous for *AdhS* and those of Kronenbourg for the *AdhF* alleles, their genotypes were used to identify partners from each mating pair. Both alleles were, however, present in the two populations, but the rare alleles (*AdhF* in Loua and *AdhS* in the brewery) were only found in heterozygous flies. Both mated and unmated individuals were genotyped to avoid any problem of identification. There were no uncertain

Table 1 Characteristics of suburban (Loua or Loukanga) and urban (Kronenbourg) populations of *Drosophila melanogaster* in Brazzaville compared to European and African populations. Data and methods used to measure the different traits are given in the references. [1]David (1982); [2]Chakir *et al*. (1996); [3]David *et al*. (1986); [4]Capy *et al*. (1993); [5]David *et al*. (1985); [6]Gibert *et al*. (1996)

Traits	Loua	Kronenbourg	Europe	Africa
Alloenzyme frequencies				
Adh(*F*)	3.03 ± 3.03 (68)	85.77 ± 3.96 (107)	94.01 ± 0.91 (1752)[1]	8.42 ± 6.03 (2518)[1]
αGpdh(*F*)	99.29 ± 0.71 (68)	90.63 ± 1.75 (88)	59.26 ± 3.69 (1476)[1]	98.83 ± 0.56 (2238)[1]
Est-6(*F*)	46.77 ± 3.23 (65)	44.38 ± 4.10 (69)	27.22 ± 1.24 (1416)[1]	62.77 ± 4.04 (1836)[1]
Est-C(*F*)	52.21 ± 2.20 (46)	88.78 ± 3.76 (107)	88.18 ± 2.27 (1438)[1]	55.12 ± 7.85 (1736)[1]
G6pd(*F*)	2.06 ± 1.00 (146)	29.98 ± 2.52 (112)	98.25 ± 0.50 (484)	0† (58)
† No replicates. Population from Lamto (Ivory Coast).				
Number of individuals in parentheses.				
Tolerance				
Ethanol	5.75 ± 0.25 (17)[2]	13.42 ± 0.41 (17)[2]	17.46 ± 0.33 (11)[3]	7.68 ± 0.39 (10)[3]
Acetic acid	5.02 ± 0.10 (17)[2]	7.06 ± 0.33 (17)[2]	11.03 ± 0.16 (12)[1]	No data available
Number of replicates in parentheses.				
Morphology (25°C)				
Females				
Abdominal bristle number	39.08 ± 0.56 (1)	41.12 ± 0.12 (1)	43.23 ± 0.29 (30)	38.74 ± 0.51 (13)
Sternopleural bristle number	16.72 ± 0.51 (1)	17.46 ± 0.43 (1)	19.57 ± 0.22 (30)	17.19 ± 0.29 (13)
Thorax length	104.00 ± 0.55 (1)	102.86 ± 0.40 (1)	107.61 ± 0.37 (30)	100.79 ± 0.73 (13)
Wing length	212.22 ± 1.61 (1)	212.09 ± 1.00 (1)	227.25 ± 0.77 (30)	203.49 ± 1.28 (13)
Ovariole number	37.01 ± 0.86 (1)	37.20 ± 1.06 (1)	44.20 ± 0.94 (30)	37.93 ± 0.90 (13)
Males				
Fresh weight	76.61 ± 0.73 (1)	77.95 ± 0.72 (1)	91.23 ± 1.55 (11)[4]	82.18 ± 1.77 (10)[4]
Abdominal bristle number	30.26 ± 0.51 (1)	38.84 ± 0.62 (1)	36.76 ± 0.52 (11)[4]	32.83 ± 0.59 (10)[4]
Sternopleural bristle number	16.32 ± 0.29 (1)	17.11 ± 0.44 (1)	18.12 ± 0.21 (11)[4]	16.76 ± 0.27 (10)[4]
Thorax length	88.65 ± 0.39 (1)	87.97 ± 0.48 (1)	94.25 ± 0.64 (11)[4]	90.39 ± 0.96 (10)[4]
Wing length	180.79 ± 0.59 (1)	184.33 ± 0.80 (1)	199.13 ± 1.57 (11)[4]	185.44 ± 3.07 (10)[4]
Number of populations in parentheses. 10 isofemale lines and 10 individuals per isofemale line were analysed.				
Pigmentation of females				
Thoracic trident (25°C)	0.01 ± 0.01 (1/18)	0.34 ± 0.04 (1/18)	0.93 ± 0.09 (11/11)[5]	0.28 ± 0.08 (7/70)[5]
Abdominal pigmentation (25°C)	16.90 ± 0.18 (1/18)	12.90 ± 0.29 (1/18)	10.60 ± 0.29 (2/20)[6]	15.70 ± 0.29 (1/10)‡
‡ Gibert and David, unpubl. results (Mt Nimba).				
Number of populations/average number of isofemale lines per population in parentheses.				
Cuticular hydrocarbons				
Females (Ratio 5,9 HD/7,11 HD)	5.46 ± 2.93 (1/48)	0.65 ± 0.57 (1/60)	close to 0 (19)§	2.66 ± 0.53 (10)¶
Males (Ratio 7P/7T)	14.11 ± 10.28 (1/42)	0.56 ± 0.54 (1/56)	0.28 ± 0.05 (19)§	3.32 ± 2.00 (15)¶
§ Rouault *et al*. (in prep.).				
¶ Rouault *et al*. (in prep.).				
Number of populations/number of individuals measured for Loua and Kronenbourg or number of populations for Europe and Africa in parentheses.				

Table 2 Genetic distances and standard deviations over six loci for microsatellites between the urban and countryside populations of *Drosophila melanogaster* from Brazzaville and three European and African populations. Distances were calculated after Nei (1987, equation 9.23)

	Malawi		France		Urban		Countryside	
Ivory Coast	0.07	0.06	0.13	0.13	0.22	0.19	0.39	0.28
Malawi			0.19	0.15	0.33	0.20	0.33	0.24
France					0.11	0.11	0.43	0.33
Urban							0.44	0.47

identifications because the frequencies of these heterozygotes are low.

In the first two types of experiments, individuals were placed in a glass vial. In the third, all individuals were placed in a bottle, 40 cm high and 15 cm in diameter. Mated individuals were removed and frozen for *Adh* genotyping. The experiment was stopped when one fourth of all possible matings had been observed.

Isolation [$II = 1 - (n_{LK} + n_{KL}/n_{KK} + n_{LL})$] (Coyne & Orr, 1989), and discrimination [$DI = -\ln(n_{LK}n_{KL}/n_{KK}n_{LL})$] indices were calculated, with n_{ij} being the number of matings between a female i and a male j. As proposed by Wu *et al.* (1995), when $DI = \infty$, because no LK mating pair was observed, a new DI value was calculated using $n_{LK} = 0.5$.

Courtship behaviour

Two traits involved in the courtship behaviour were analysed. The inter pulse interval (IPI) and the cuticular hydrocarbons. The IPI, a characteristic of the male love song (Kyriacou & Hall, 1985), were measured at 25°C according to the method described by Paillette *et al.* (1997). For this trait, individuals 3–5 days old were used. Hydrocarbon levels, which can be used as population markers, particularly to discriminate between African and European flies, were analysed according to Antony *et al.* (1985) on a Girdel 300 chromatograph with a Cpsil 25 m capillary column. Virgin flies 4–6 days old were used.

Results

Population differentiation

Table 1 compares the countryside and the urban populations with the average characteristics of African and European populations. The *AdhF* allele was frequent in the urban population as in Europe. The same was found for *Est-C*. The urban strain was closer to African populations for αGpdh, and intermediate between European and African populations for *G6pd*. The two Brazzaville populations were intermediate between the European and African averages for *Est-6*.

The countryside population was in Hardy–Weinberg equilibrium for *Adh*, because the fixation index (calculated using GENEPOP, Raymond & Rousset, 1995) was close to 0 ($F_{IS} = -0.03$), whereas the F_{IS} values varied greatly over time for the urban population (0.05 in 1988 and 0.30 in 1989, P-values are 0.429 and 0.005, respectively). Similarly, departure from Hardy–Weinberg equilibrium is significant only for the *Adh* in 1989. In an organism like *Drosophila*, significant F_{IS} can originate either from population mixing ('Wahlund effect'), from homogamy, or from a selective disadvantage of heterozygotes. All these phenomena suggest some mixing of individuals from breweries with those from neighbouring populations which have characteristics similar to those from the countryside.

Differentiation for microsatellite variation is shown in Table 2. The urban population from Brazzaville was genetically very similar to the European population from France ($D = 0.11$, SD = 0.11), but very unlike the Brazzaville countryside population ($D = 0.44$, SD = 0.47). A similar value was found between the countryside and French populations (0.43, SD = 0.33). Other African populations from Ivory Coast and Malawi (Michalakis & Veuille, 1996) were substantially different from the two Brazzaville populations. In particular, the countryside population seems to be distantly related to the other African populations. This is mainly because of a private allele present in Loua and absent from all the other populations including the urban population of Brazzaville. This may only indicate genetic differentiation in the African continent, in agreement with other studies (Lemeunier *et al.*, 1994). When the private allele of Loua is removed, distances between the countryside and French populations and between urban and French populations were again not significantly different (0.34 SD = 0.29 vs. 0.33 SD = 0.43).

The urban population was about two times more ethanol tolerant than that of the countryside (13.42% vs. 5.75%), but consistently less tolerant than the European populations (13.42% vs. 17.46%). The

morphological characteristics of the urban population were similar to those of the countryside for both sexes. Finally, the abdominal and thoracic pigmentation scores in females were intermediate between the African and European populations (Table 1).

Mating choice experiments

Significant homogamy was detected in all the experiments (Table 3). KK mating pairs were more frequent than expected, and LK pairs less frequent. Countryside males tended to mate in equal proportions with both kinds of females (45% vs. 55%) in experiments involving one male and two females, whereas urban males copulated more frequently with urban females than with other females (79% vs. 21%). The experiments involving one female and two males showed that countryside females copulated more frequently with countryside males (81% vs. 19%) whereas urban females copulated more frequently with urban males (85% vs. 15%). The observed values for all the behavioural experiments were significantly different from random mating, confirming preliminary results (Paillette *et al.*, 1993). The only exception was the absence of mating preference of males from Loua.

The choice experiments involving four individuals gave an isolation index between the two populations from Brazzaville of 0.77, and of 0.55 between the countryside population and European strain from Bordeaux (France). Differences in discrimination index (2.90 ± 0.06 vs. 2.35 ± 0.06) were also observed, suggesting greater sexual isolation between sympatric populations than between allopatric ones (Table 3).

Courtship behaviour

The latency to the first copulation was shorter for KK experimental pairs (11 ± 2 min, $n = 73$) than for LL pairs (17 ± 3, $n = 17$), but was much longer for LK pairs (28 ± 5, $n = 4$), in agreement with published results (Cohet & David, 1980). The inter pulse interval (IPI) differed significantly in the two populations (40.19 ± 0.18 milliseconds, number of individuals = 32 and number of measures = 512 IPIs for L; and 38.82 ± 0.20 milliseconds, number of individuals = 30 and number of measures = 505 for K; $P < 0.001$), but IPIs may vary among populations, independently of their geographical origin (Jallon, unpubl. results).

Drosophila melanogaster is sexually dimorphic for cuticular hydrocarbons. Female compounds elicit specific steps in the male courtship. The cuticular

Table 3 Mating choice experiments using three, four and more individuals of *Drosophila melanogaster*

a Mating choice between Loua (L) and Kronenbourg (K)

Individuals in the mating chamber												
Females		Males		Number of mating pairs observed								
K	L	K	L	KK	KL	LK	LL	*n*	χ^2	*P*	II	DI
1	1	1		27		7		34	11.76	< 0.001	0.74	
1	1		1		18		22	40	0.40	Ns	0.18	
1		1	1	22	4			26	12.46	< 0.001	0.82	
	1	1	1			6	26	32	12.50	< 0.001	0.77	
1	1	1	1	73	17	4	17	111	32.01	< 0.001	0.77	2.90 ± 0.06
50	50	50	50	11	8	0	7	1	7.09	< 0.01	0.59	2.96 ± 0.30

b Mating choice between Loua (L) and Bordeaux (B)

Individuals in the mating chamber												
Females		Males		Number of mating pairs observed								
B	L	B	L	BB	BL	LB	LL	*n*	χ^2	*P*	II	DI
1	1	1	1	69	35	3	16	123	16.91	< 0.001	0.55	2.35 ± 0.06

Individuals were placed in empty glass vials. First mating pairs were isolated and individuals were characterized by their *Adh* type. K, Kronenbourg; L, Loua; B, Bordeaux. II, Isolation Index, is 1 – (number of heterogamic pairs/number of homogamic pairs); DI, Discriminant Index and its SE (see Wu *et al.*, 1995 for more details); as proposed by these authors, when DI = ∞, because no LK mating pair was observed, a new DI value was calculated using $n_{LK} = 0.5$; *n*, number of trails. The multichoice experiment with 100 individuals (50 males and 50 females) of each populations was stopped when the number of successful mating pairs reached 25%. Individuals were identified using *Adh* alleles as markers.

hydrocarbons in a sample of world populations, including many African strains have been described (Scott & Richmond, 1988; Ferveur *et al*., 1996). The distribution is bimodal. African populations differ from non-African populations in the ratios of two compounds: 5,9-Heptacosadiene (5,9HD) and 7,11-Heptacosadiene (7,11HD) in females, and 7 Pentacosene (7P) and 7 Tricosene (7T) in males. The 5,9HD/7,11HD ratio of females and the 7P/7T ratio of males were close to 0 in the urban population (Table 1), whereas those of individuals from the countryside were higher (5.46 and 14.11, respectively). Comparison of Loua and Kronenbourg for female and male components shows significant differences ($P < 0.001$). The urban Brazzaville population was typically European and the countryside populations were typically African.

Discussion

Our results show that there are clearly two population types in the Brazzaville area over a microgeographical scale. They differ in a number of traits, including microsatellite and allozyme frequencies (except for *Est-6*), ethanol tolerance, abdominal and thoracic pigmentation, cuticular hydrocarbons, and behaviour. The only exceptions are morphological traits, for which both populations are typically African. Resistance to ethanol may be linked to the *Adh* genotype, but all the other traits are probably genetically independent.

Great homogamy was detected in laboratory conditions. Sexual isolation probably occurs in natural conditions, as suggested by the high F_{IS} values in the urban areas. Thus, urban and suburban populations seem to coexist but there is little genetic exchange. This unusual situation was initially observed for *Adh* (Vouidibio *et al*., 1989), and is now extended to other traits, raising the question of the origin of these populations.

Possible origin of the urban population

Ever since it was first suggested that *D. melanogaster* was of African origin (Lachaise *et al*., 1988 and references therein), genetic studies have indicated that this species spread to Europe, then to other temperate countries of the world, after adapting to man-modified environments (David & Capy, 1988; Lachaise *et al*., 1988). Temperate populations are better adapted to alcohol: they are more resistant to ethanol and show high frequencies of the *Adh-F* allele. The possibility of these temperate populations spreading back to Africa was never considered, except for Ivory Coast, where the *Adh-F* allele could be of European origin (Veuille *et al*., 1998). The periurban Brazzaville population is very like other African populations, and we can assume that it is a genuine native population. There are two explanations for the urban population. It could result from a habitat choice leading to selection for ethanol tolerance in populations native to Congo. Isolation would thus be purely ecological. Or it could be the result of migration from an allopatric population that had different genetic properties. In this case, isolation would originally be allopatric.

The microsatellites and cuticular hydrocarbons suggest that the urban population was introduced from Europe, or a European-like population. Breweries are stable habitats that support permanent populations adapted to an alcohol-rich environment. Because the raw materials used to make beer in Brazzaville are imported from Europe, such imports might have been responsible for the *D. melanogaster* invasion.

The only inconsistent result is the morphological similarity of the two African populations. This might result from a secondary adaptation of imported European flies to a tropical environment. Morphological traits can adapt rapidly to a local environment, and the European migrants could have evolved African-like adaptations quite recently. Similar morphological changes were observed following the introduction of *D. simulans* to Japan (Watada *et al*., 1986) and of *D. subobscura* to Chile (Budnick *et al*., 1991).

The maintenance of a high frequency of the *AdhF* allele associated with ethanol tolerance is probably caused by selective pressures imposed by the ecological niche of this introduced population. The intermediate value of other traits between Africa and Europe could result from lower selective forces or from mixing with surrounding native populations. The persistence of large 'European-like' populations in breweries may also explain the strong differentiation of traits related to sexual choice, because of the disadvantage of matings producing hybrid progeny.

Incipient speciation?

Our results are somewhat similar to those obtained in Zimbabwe, where several strains are sexually isolated, both from African strains and from European and American strains (Wu *et al*., 1995; Hollocher *et al*., 1997a,b). However, there is no indication that the Zimbabwe strains have consistent differences for other genetic factors. It would be interesting to compare their genetical characteristics with those of populations from Congo and Europe to determine whether the phenomena are similar.

The situation in Brazzaville can be considered as a 'natural' experiment involving the confrontation of two populations which had previously been geographically

isolated over thousands of years (David & Capy, 1988). Differences in male sexual activity have been previously detected in African and European strains of *D. melanogaster* (Cohet & David, 1980). Along with the differences in cuticular hydrocarbons, these traits are probably involved in the present partial prezygotic isolation between the two types of populations. The higher isolation index between sympatric than between allopatric populations is in agreement with the classic "allopatric" model of speciation involving the local reinforcement of differences that initially evolved in allopatry (see for review Rice & Hostert, 1993; Butlin, 1995; Coyne & Orr, 1997).

The status of natural Afrotropical populations

Our results suggest that the sexual isolation between a Zimbabwean and North American populations (Wu *et al*., 1995; Hollocher *et al*., 1997a,b) is not restricted to this African population, but might be a general feature of Afrotropical populations. This is suggested not only by the Brazzaville observations, but also by the widespread occurrence of specific cuticular pheromones in Africa (Jallon & David, 1987).

Many genetic investigations have shown that African populations probably represent the ancestral populations of the species. Therefore, it would be interesting to determine whether they are sufficiently differentiated from temperate populations, to be considered as a taxonomic entity, so that local coexistence in sympatry should be possible at least when different resources/habitats are available.

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References

ANTONY, C., DAVIS, T. L., CARLSON, D. A., PÉCHINÉ, J. M. AND JALLON, J. M. 1985. Compared behavioral responses of male *Drosophila melanogaster* (Canton S) to natural and synthetic aphrodisiacs. *J. Chem. Ecol*., **11**, 1617–1629.

BUDNICK, M., CIFUENTES, C. AND BRNCIC, D. 1991. Quantitative analysis of genetic differentiation among European and Chilean strains of *Drosophila subobscura*. *Heredity*, **67**, 29–33.

BUTLIN, R. 1995. Reinforcement: an idea evolving. *Trends Ecol. Evol*., **10**, 432–434.

CAPY, P., PLA, E. AND DAVID, J. R. 1993. Phenotypic and genetic variability of morphological traits in natural populations of *Drosophila melanogaster* and *D. simulans*. I. Geographic variations. *Génét. Sél. Évol*., **25**, 517–536.

CHAKIR, M., CAPY, P., GÉNERMONT, J., PLA, E. AND DAVID, J. R. 1996. Adaptation to fermenting resources in *Drosophila melanogaster*: ethanol and acetic acid tolerances share a common genetic basis. *Evolution*, **50**, 767–776.

COHET, Y. AND DAVID, J. R. 1980. Geographic divergence and sexual behaviour: comparison of mating systems in French and Afrotropical populations of *Drosophila melanogaster*. *Genetica*, **54**, 161–165.

COYNE, J. A. AND ORR, H. A. 1989. Pattern of speciation in *Drosophila*. *Evolution*, **43**, 362–381.

COYNE, J. A. AND ORR, H. A. 1997. 'Pattern of speciation in *Drosophila*' revisited. *Evolution*, **51**, 295–303.

DAVID, J. R. 1982. Latitudinal variability of *Drosophila melanogaster*: allozyme frequencies divergence between European and Afrotropical populations. *Biochem. Genet*., **20**, 747–761.

DAVID, J. R. AND CAPY, P. 1988. Genetic variation of *Drosophila melanogaster* natural populations. *Trends Genet*., **4**, 106–111.

DAVID, J. R., CAPY, P., PAYANT, V. AND TSAKAS, S. 1985. Thoracic trident pigmentation in *Drosophila melanogaster*: differentiation of geographical populations. *Génét. Sél. Évol*., **17**, 211–224.

DAVID, J. R., MERCOT, H., CAPY, P., MCEVEY, S. F. AND HERREWEGE, J. V. 1986. Alcohol tolerance and *Adh* gene frequencies in European and African populations of *Drosophila melanogaster*. *Genet. Sel. Évol*., **18**, 405–416.

FERVEUR, J. F., COBB, M., BOUKELLA, H. AND JALLON, J.-M. 1996. World-wide variation in *Drosophila melanogaster* sex pheromone: behavioural effects, genetic bases and potential evolutionary consequences. *Genetica*, **97**, 73–80.

GIBERT, P., MORETEAU, B., MORETEAU, J.-C. AND DAVID, J. R. 1996. Growth temperature and adult pigmentation in two *Drosophila* sibling species: an adaptive convergence of reaction norm in sympatric populations? *Evolution*, **50**, 2346–2353.

HENDERSON, N. R. AND LAMBERT, D. M. 1982. No significant deviation from random mating of wordwide populations of *Drosophila melanogaster*. *Nature*, **300**, 361–375.

HOLLOCHER, H., TING, C.-I., POLLACK, F. AND WU, C.-I. 1997a. Incipient speciation by sexual isolation in *Drosophila melanogaster:* variation in mating preference and correlation between sexes. *Evolution*, **51**, 1175–1181.

HOLLOCHER, H., TING, C.-T., WU, M.-L. AND WU, C.-I. 1997b. Incipient speciation by sexual isolation in *Drosophila melanogaster:* extensive genetic divergence without reinforcement. *Genetics*, **147**, 1191–1201.

JALLON, J. M. AND DAVID, J. R. 1987. Variations in cuticular hydrocarbons among the eight species of the *Drosophila melanogaster* subgroup. *Evolution*, **41**, 294–302.

KYRIACOU, C. P. AND HALL, J. C. 1985. Action potential mutations stop a biological clock in *Drosophila*. *Nature*, **314**, 171–173.

LACHAISE, D., CARIOU, M. L., DAVID, J. R., LEMEUNIER, F., TSACAS, L. AND ASHBURNER, M. 1988. Historical biogeography of the *Drosophila melanogaster* species subgroup. *Evol. Biol*., **22**, 159–225.

LEMEUNIER, F., AULARD, S., BÉNASSI, V. AND VEUILLE, M. 1994. Fruitfly origins. *Nature*, **371**, 25–25.

MICHALAKIS, Y. AND VEUILLE, M. 1996. Length variation of CAG/CAA trinucleotide repeats in natural populations of *Drosophila melanogaster* and its relation to the recombination rate. *Genetics*, **143**, 1713–1725.

NEI, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.

PAILLETTE, M., BIZAT, N. AND JOLY, D. 1997. Differentiation of dialects and courtship strategies in allopatric populations of *Drosophila teissieri*. *J. Insect Physiol.*, **43**, 809–814.

PAILLETTE, M., CAPY, P., PLA, E. AND DAVID, J. R. 1993. Isolement reproducteur par homogamie entre deux 'races d'habitat' sympatriques chez *Drosophila melanogaster*. *Rev. Ecol. (Terre Vie)*, **48**, 229–238.

RAYMOND, M. AND ROUSSET, F. 1995. GENEPOP: population genetics software for exact tests and ecumenicism. *J. Hered.*, **86**, 248–249.

RICE, W. R. AND HOSTERT, E. E. 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution*, **47**, 1637–1653.

SCOTT, D. AND RICHMOND, R. C. 1988. A genetic analysis of male-predominant pheromones in *Drosophila melanogaster*. *Genetics*, **119**, 639–646.

TREGENZA, T. AND BRIDLE, J. R. 1997. The diversity of speciation. *Trends Ecol. Evol.*, **12**, 382–383.

VEUILLE, M., BENASSI, V., AULARD, S. AND DEPAULIS, F. 1998. Allele-specific population structure of *Drosophila melanogaster* alcohol dehydrogenase at the molecular level. *Genetics*, **149**, 971–981.

VOUIDIBIO, J., CAPY, P., DEFAYE, D., PLA, E., SANDRIN, E., CSINK, J. *ET AL*. 1989. Short-range genetic structure of *Drosophila melanogaster* populations in an Afrotropical urban area and its significance. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 8442–8446.

WATADA, M., OHBA, S. AND TOBARI, Y. N. 1986. Genetic differentiation in Japanese populations of *Drosophila simulans* and *D. melanogaster*. *Jap. J. Genet.*, **61**, 469–480.

WU, C.-I., HOLLOCHER, H., BEGUN, D. J., AQUADRO, C. F., XU, Y. AND WU, M. L. 1995. Sexual isolation in *Drosophila melanogaster*: a possible case of incipient speciation. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 2519–2523.