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Gene Flow and Divergence under Disruptive Selection

Abstract. Two halves of a population exposed to selection in opposing directions can diverge despite gene flow of the same amount as is given by random mating. Divergence was as great as it is with complete isolation. Isolation, therefore, is not a prerequisite of divergence under divergent selection pressures.

Thoday and Boam (*1*, *2*) have demonstrated that two halves of a population of *Drosophila melanogaster* can diverge when selected for opposite extreme values of a metric character (sternopleural chaeta number) even though all the individuals of every generation are the progeny of hybridization between the two halves. They have pointed out that their results throw doubt on the assumption that isolation is a necessary prerequisite of such divergence in natural populations.

The population maintained by Thoday and Boam involved forced gene flow such that the two halves of the population exchanged 50 percent of their genes in each generation. This is twice as much gene flow as the maximum usually considered in relation to natural populations, for random mating involves only 25 percent gene flow. We have accordingly run two selection lines under disruptive selection with positive assortative mating, using a system that gives 25 percent gene flow by male migration (Table 1). For comparative purposes, two lines with 50 percent gene flow (which differed from that of Thoday and Boam in that males were used for migration), and two with no gene flow (complete isolation) were maintained. All originated from the wild stock that was used by Thoday and Boam (*1–3*). Culture conditions and the proportion of flies selected were also the same.

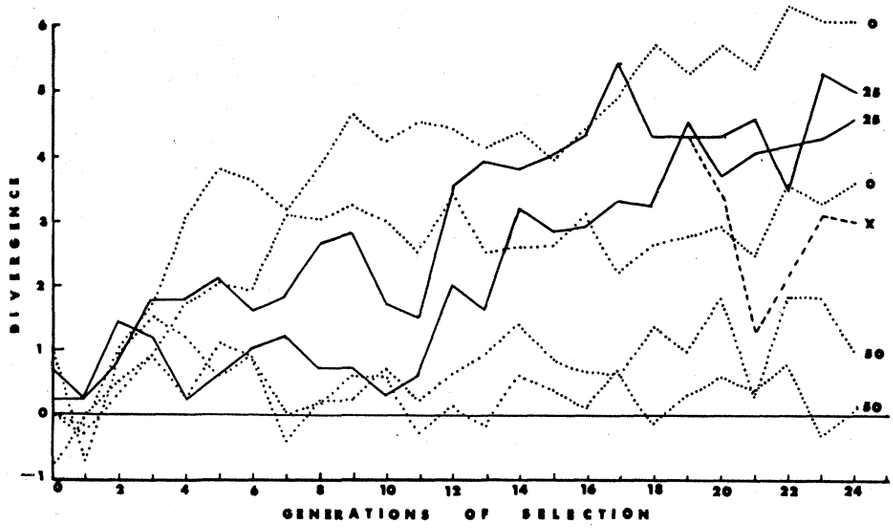

Fig. 1. Differences, in chaetae per fly, between the mean for the high half and that for the low half of each line in each generation. Dotted curves, the 0 and 50 percent gene-flow lines. Solid curves, the 25 percent gene-flow lines. Broken curve (*X*), the 25 percent gene-flow negative-assortative-mating subline.

The results are presented in Fig. 1, in which the differences between the mean chaeta number of the high and that of the low halves of each line are plotted for each generation.

Fifty percent gene flow permitted some divergence, though it is clearly a great restriction. The divergences of the 25 percent gene flow populations are very much greater. Though slower to develop than those permitted by complete isolation (no gene flow), their magnitude is of the same order. This might not be so if the population sizes were larger, but it provides a striking demonstration that very considerable divergence is possible without isolation. The mean chaeta numbers of these populations are of the order of 19, and the differences between the high and low halves of the 25 percent gene flow populations must be considered very large in relation to this mean.

The relevance of these results to theories related to natural populations is somewhat limited by the fact that the flies which convey genes from one half of a population to the other are selected for chaeta numbers deviating in the direction appropriate to the half population *to* which they are made to migrate. We have therefore taken a subsidiary line from one of the 25 percent gene flow populations and maintained it under 25 percent gene flow, disruptive selection with negative assortative mating. That is to say, the flies that are to carry genes from the low- to the high-chaeta-number half of the population are selected for low chaeta number, and those that migrate from high to low halves are selected for high

Table 1. Mating and selection system used for disruptive selection with 25 percent gene flow. The entries designate the flies chosen to perpetuate each of four female lines in each generation. H indicates the highest, and L the lowest chaeta number fly found in the appropriate culture. The letters A, B, C, and D indicate the culture from which the fly was selected.

Female line	Female parent	Male parent, Generation 1	Male parent, Generation 2	Male parent, Generation 3	Male parent, Generation 4
		High half population			
A	AH	BH	DH	BH	DH
B	BH	CH	AH	CH	AH
		Low half population			
C	CL	DL	BL	DL	BL
D	DL	AL	CL	AL	CL

chaeta number. The results are also illustrated in Fig. 1. The change of selection system, of course, reduced the difference of mean between the two halves of the population. But a large difference has been retained. There can therefore be no doubt that a considerable difference could be maintained under random mating.

These selection experiments therefore demonstrate that it is in principle possible for ecotypes or biological races to diverge under the divergent selection pressures that might be imposed in heterogeneous habitats. The concept that random mating must "swamp" the genetic differences involved is not sound. Isolation barriers may be involved in much, even most, evolutionary divergence, but they are not a prerequisite of such divergence (*4*).

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Enzymatic O-Methylation of N-Acetylserotonin to Melatonin

Abstract. An enzyme, hydroxyindole-O-methyl transferase, that can transfer the methyl group of S-adenosylmethionine to the hydroxy group of N-acetylserotonin to form the hormone melatonin is described. This enzyme, which is highly localized in the pineal gland, also O-methylates serotonin.

Recently Lerner and co-workers (*1*) isolated a new hormone, melatonin (N-acetyl-5-methoxytryptamine), from the pineal gland and peripheral nerves of man, monkey, and cow. This compound was found to lighten the color of frog melanocytes and block the actions of the melanocyte-stimulating and adrenocorticotropic hormones (*1*). McIsaac and Page have recently shown that serotonin (5-hydroxytryptamine) is converted to N-acetylserotonin in vivo (*2*). We wish to report the isolation of an enzyme that forms melatonin by the O-methylation of N-acetylserotonin.

Table 1. Enzymatic O-methylation of N-acetylserotonin to melatonin. The soluble supernatant fraction obtained from 16 mg of cow pineal gland was incubated at 37°C with 0.1 μmole of N-acetylserotonin, 100 μmoles of phosphate buffer (*p*H 8.0), and 0.1 μmole of S-adenosylmethionine. After 2 hours' incubation melatonin was determined in the incubation mixture (*4*).

System	Melatonin formed (mμmoles)
Complete system	11
S-adenosylmethionine omitted	0

Since melatonin was found to be highly localized in the pineal gland (*1*), this tissue was examined for the presence of an enzyme that could O-methylate hydroxyindoles. Pineal glands from cows (*3*) were homogenized with ice-cold isotonic potassium chloride and centrifuged at 78,000*g*. The resulting soluble supernatant fraction was incubated with N-acetylserotonin and S-adenosylmethionine at *p*H 8.0. After a 2-hour incubation at 37°C the reaction product was extracted from the incubation mixture with chloroform and the organic phase was washed with water to remove residual substrate. The chloroform extract was then evaporated to dryness in a stream of warm air and the residue taken up in 3*N* HCl. A fluorescent metabolite was found to be present in the acid extract with a maximum fluorescent peak in 3*N* HCl at 540 mμ upon activation at 310 mμ; this is characteristic of 5-hydroxy- and 5-methoxyindoles. This metabolite had the same fluorescent spectrum, the same R_f values in butanol, acetic acid, and water (100 : 35 : 70) (0.91) and in N-propanol and 1*N* ammonia (5 : 1) (0.89), and the same color reactions and partition coefficient as authentic melatonin. When S-adenosylmethionine was omitted from the incubation mixture, no melatonin was formed (Table 1). These observations demonstrate the existence of an enzyme (hydroxyindole-O-methyl transferase) that can transfer the methyl group of S-adenosylmethionine to the hydroxy group of N-acetylserotonin. The reaction is shown in Fig. 1.

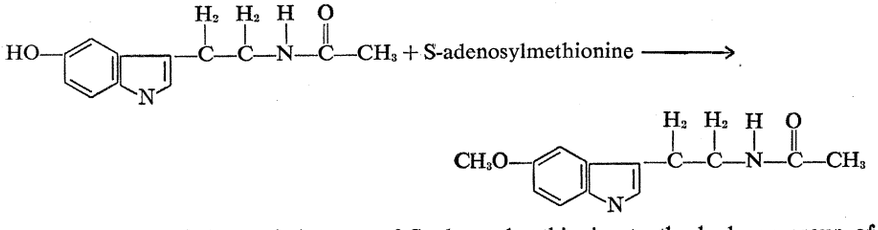

Fig. 1. Transfer of the methyl group of S-adenosylmethionine to the hydroxy group of N-acetylserotonin.

Hydroxyindole-O-methyl transferase has been purified about 20-fold from beef pineal gland by heat treatment, ammonium sulfate fractionation, and adsorption and elution from alumina Cγ gel (*4*). Unlike catechol-O-methyl transferase (*5*), the enzyme has no requirement for Mg^{++}. It could not be detected in liver and kidney of a number of mammalian species, but was found in the pineal gland of the monkey (*4*). The lack of the requirement for Mg^{++} and the unique localization of hydroxyindole-O-methyl transferase indicates that it is different from catechol-O-methyl transferase (*5*) and the other known transferases (*6, 7*).

Incubation of serotonin with hydroxyindole-O-methyl transferase and S-adenosylmethionine resulted in the formation of a product having the characteristics of authentic 5-methoxyserotonin. However, the rate of O-methylation of serotonin was only one-tenth that of N-acetylserotonin (*4*). This finding suggests that acetylation precedes O-methylation in the formation of melatonin as follows:

Serotonin ⟶ N-acetylserotonin ⟶ melatonin

From the results described in this report and elsewhere, it is becoming increasingly apparent that O- and N-methyltransferases (*5–7*) requiring S-adenosylmethionine are playing key roles in the biosynthesis and inactivation of biologically active amines and their derivatives (*8*).

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