

# ADDITIONAL EVIDENCE ON SEXUAL ISOLATION WITHIN *DROSOPHILA BIRCHII*

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Sexual isolation and hybrid sterility are primary mechanisms of reproductive isolation in speciation processes. These components of speciation are frequently observed among closely related species in the genus *Drosophila* (Patterson and Stone, 1952). Investigation of isolating mechanisms has disclosed the existence of races or incipient species in a number of temperate *Drosophila* species, e.g., as reviewed by Dobzhansky (1963).

An interesting situation is encountered in the Australasian species *Drosophila birchii*, a sibling species of *D. serrata*. This species is apparently actually a complex of reproductively and genetically isolated groups. The existence of sexual isolation between *D. birchii* strains of different geographic origins has been previously reported by Ayala (1965). These geographic strains are indistinguishable morphologically, although the southern strain (Cairns) appears to be darker in coloration of female abdomen than the others. From a cytological point of view, however, it has been demonstrated that *D. birchii* varies greatly as to the X, Y, and 4th chromosome morphology (Baimai, 1969). The Rabaul and Cairns populations have been found to be uniformly characterized by  $X_4Y_2$  sex chromosomes while the strain from Daru (an island off the coast of Papua) exhibits a unique small J-shaped Y-chromosome,  $Y_3$ , accompanied by chromosome  $X_1$ . In the Papua-New Guinea strains, on the other hand, chromosome  $X_1$  is widespread and two other less commonly found chromosomes  $X_2$  and  $X_3$  are ac-

companied by a common Y-chromosome,  $Y_1$ . The existence of such characteristic sex chromosome types in *D. birchii* seems to add weight to the results of sexual isolation tests performed by previous workers.

In relation to reproductive isolation between the *D. birchii* strains, two additional strains from Daru and Wewak (on the north coast of New Guinea) have been discovered and are employed in this study. The Daru strain is of particular interest since it exhibits not only uniqueness of a Y-chromosome type and certain gene arrangements but also forms part of a seemingly interesting "chain of races or subspecies." The outcome of these experiments arranged to determine reproductive isolation between the geographic strains of *D. birchii* is reported in the present article.

## METHODS AND RESULTS

The seven *D. birchii* strains of different geographic origins employed in this study are Rabaul, Cairns (each with  $X_4Y_2$  sex chromosomes); Port Moresby, Bulolo, Popondetta, Wewak (with  $X_1Y_1$  sex chromosomes in each case); and Daru (characterized by  $X_1Y_3$  sex chromosomes) (Fig. 1). Each of these stocks was established from individual wild-caught females which were already inseminated prior to capture, and the stocks have been maintained in the laboratory for 1 to 2 years. Two series of experiments were carried out as follows:

The first series of experiments consisted of systematic intercrosses between the different geographic strains. The method employed here was adapted from the no-choice technique used by Dobzhansky and

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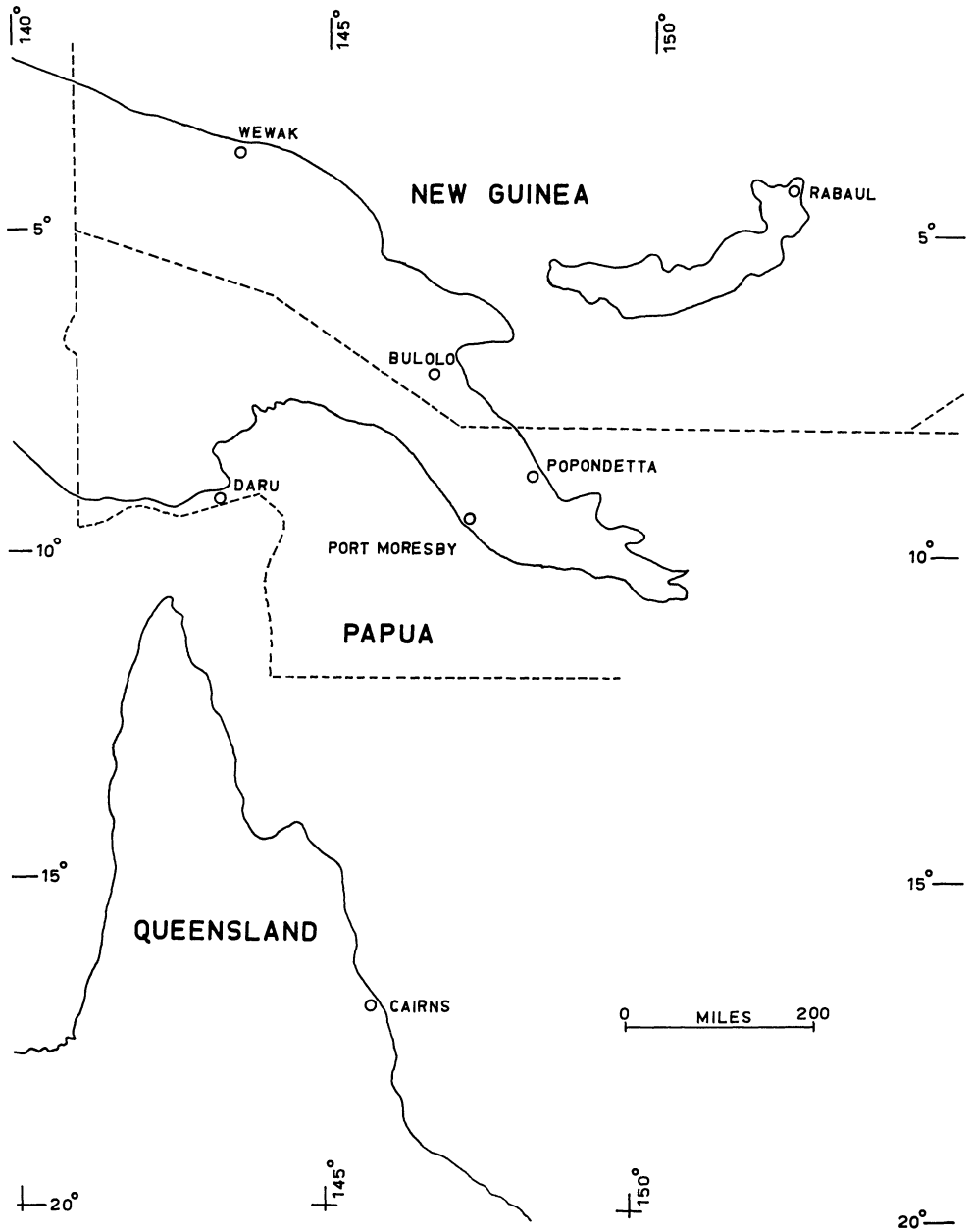


FIG. 1. Geographic origin of the strains of *Drosophila birchii*.

Spassky (1959). In *D. birchii*, all intercrosses proceeded successfully and yielded  $F_1$  hybrids. Fertility tests on the  $F_1$  hybrid offspring were made by backcrossing the hybrids to the parental strains.

Failure of backcrosses to produce offspring in some cases was due to sterility of  $F_1$  male hybrids, as has been confirmed by microscopic examination of the male hybrid testes, which produced no motile

MALES FEMALES	RABAU	CAIRNS	DARU	MORESBY	BULOLO	POPONDETTA	WEWAK
RABAU		SM	SM	SM	SM	SM	SM
CAIRNS	SM		F	F	F	F	F
DARU	SM	F		F	F	F	F
MORESBY	SM	SM	F		F	F	F
BULOLO	SM	SM	F	F		F	F
POPONDETTA	SM	SM	F	F	F		F
WEWAK	SM	SM	F	F	F	F	

FIG. 2. Summary of the results of intercrosses between *D. birchii* strains of different geographic origins. SM = sterile male  $F_1$  hybrids; F = fertile hybrids.

sperm. The results of all the possible combinations of intercrosses are summarized in Figure 2; the sterile male hybrids are symbolized by SM and the fertile hybrids of both sexes are marked by F.

As may be seen from Figure 2, intercrosses having females from Rabaul or males from Cairns produced sterile  $F_1$  male hybrids except for the intercrosses involving Cairns and Daru strains which yielded fertile hybrids in both directions. Intercrosses between the geographic strains of the Papua-New Guinea area including Daru proceeded quite easily and yielded fertile hybrids of both sexes. This is a

very interesting situation suggesting the possibility that the Daru strain could perform an important role in bridging the gap and allowing gene flow between the populations of Papua-New Guinea and Cairns. Gene exchange between these populations would almost certainly occur naturally, were these two territories not separated by the geographic barrier of Torres Strait. In fact, there is no evidence as yet to indicate any interbreeding in nature between these populations. It thus appears, at this stage, that the constitution of the Daru strain is that of a transitional form.

TABLE 1. *Sexual preferences among the geographic strains of D. birchii. The first number in each column refers to percent homogamic matings; the second number to percent heterogamic matings. Numbers of females dissected are about 100 for each cross.*

♂♂ ♀♀	Rabaul	Cairns	Daru	Wewak	Moresby	Bulolo	Popondetta
Rabaul		92-72**	97-78**	99-79**	90-45**	96-24**	97-80**
Cairns	99-80**		100-35**	98-90*	96-93	96-80**	99-80**
Daru	97-11**	93-67**		94-84*	91-85	94-75**	94-68**
Wewak	90-24**	84-72	89-84		89-86	84-82	87-67**
Moresby	92-26**	90-74*	89-93	91-90		99-94	97-81**
Bulolo	86-22**	69-86*	80-94*	67-80	90-91		95-77**
Popondetta	90-14**	75-93*	79-94*	84-96*	77-88	84-96*	

\*  $P < 0.05$

\*\*  $P < 0.001$

The second series of experiments was performed in order to determine whether there were any mating preferences between strains of *D. birchii* of different geographic origins. The male-choice method, which involves groups of one kind of males confined with two kinds of females (one from the same, one from a different locality) for a certain period of time, was employed in this sexual isolation test. The experiments were arranged as follows: virgin females and males were selected and aged for about 8-10 days in isolation. Ten females of each of the two geographic strains and 10 males of one of the strains were placed in a half-pint culture bottle. Since different geographic strains of *D. birchii* are morphologically indistinguishable, females of one of the strains tested were marked by clipping a part of one of their wings. After 24 hours of exposure

to the males, sexes were separated. All females were then dissected in 0.7% saline solution and ventral receptacles extracted. The examinations for the presence or absence of spermatozoa in the receptacles were made by observation under a light microscope (bright-field illumination). In every cross, at least 10 repeated sets of matings were performed. Numbers of homogamic and heterogamic inseminations in each cross were recorded and calculated as percentages of insemination. The observed differences between the two types of matings are compared by Chi-square tests (by means of a "two by two" table) for any statistically significant mating preferences (Table 1). The isolation indices were calculated from Stalker's formula (Stalker, 1942).

Data on the 42 possible combinations are given in Tables 1 and 2. It may be seen that Rabaul females were infre-

TABLE 2. *Sexual isolation among strains of D. birchii. The figures given represent the isolation indices. Numbers of females dissected are about 100 for each cross.*

♂♂ ♀♀	Rabaul	Cairns	Daru	Wewak	Moresby	Bulolo	Popondetta
Rabaul		0.12	0.11	0.11	0.33	0.60	0.10
Cairns	0.11		0.48	0.04	0.02	0.09	0.11
Daru	0.80	0.16		0.06	0.04	0.11	0.16
Wewak	0.58	0.08	0.03		0.02	0.01	0.13
Moresby	0.56	0.10	0.03*	0.01		0.03	0.09
Bulolo	0.59	0.11*	0.08*	0.09*	0.01*		0.11
Popondetta	0.73	0.11*	0.09*	0.07*	0.07*	0.07*	

\* Negative value.

quently inseminated by males of the Papua-New Guinea strains. Thus isolation indices are high, ranging from 0.56 to 0.80.

On the contrary, the reciprocal inseminations involving males from Rabaul occurred fairly frequently, indicating that Rabaul males were accepted quite readily by both kinds of females. Thus the isolation indices are lower, ranging from 0.10 to 0.33, with the possible exception of Rabaul male—Bulolo female ( $I = 0.60$ ), but they are statistically significant regarding mating preferences. It thus appears that gene flow would be somewhat greater from the Rabaul strain into Papua-New Guinea strains than in the reverse direction if interbreeding between these populations in nature could occur. It may be noted that the data are quite consistent in sexual isolation between the Rabaul strain and Papua-New Guinea strains. Moreover, matings between Bulolo and Rabaul, and Moresby and Rabaul showed rather high isolation indices in both directions. Mating preference seems to exist to a slight extent between Rabaul and Cairns strains, since about equal isolation indices were obtained (with Rabaul males  $I = 0.12$ ; with Cairns males  $I = 0.11$ ).

Strangely enough, females of Cairns, Daru, Bulolo, and Wewak apparently accepted the males of Popondetta more readily than did Popondetta's own females. Consequently, they give negative values for isolation indices and are statistically significant. A possible hypothesis is that Popondetta females are less sexually active than other females. Likewise, significantly negative values of isolation indices were obtained in the Bulolo-Daru and Bulolo-Cairns crosses, each of which involved Bulolo males. Other isolation indices with negative values were also observed but they are not significant.

Crosses between Daru and Cairns strains showed significant preferences for homogamic matings in both directions. The isolation index is considerably higher in

the mating involving Cairns males ( $I = 0.48$ ) than in the reciprocal cross ( $I = 0.16$ ). According to the data, 24 matings out of the 42 possible combinations showed statistically significant preferences for homogamic matings, while six crosses exhibited preferences for heterogamic matings, based on the figures that are statistically significant. The other 12 crosses showed no evidence of mating preferences.

#### CONCLUSIONS AND DISCUSSION

The Rabaul strain is reproductively isolated from all of the others in the sense that it exhibits a high degree of sexual isolation from the other strains and is further reinforced by male hybrid sterility. The Cairns strain, on the other hand, is partially isolated reproductively from those in Papua-New Guinea, with the possible exception of the Daru strain. Thus the present data are correlated with the results obtained by Dobzhansky and Mather (1961), and Ayala (1965). Even more remarkable, however, the Daru strain is capable of interbreeding with the Cairns and Papua-New Guinea strains with ease under laboratory conditions, yielding fertile hybrids. This striking feature in *D. birchii* resembles the situation in *D. paulistorum*. It thus seems warranted to conclude that *D. birchii* is a polytypic species partly divided within itself into reproductively and genetically divergent races.

A part of the sterility of male hybrids in *D. birchii* is apparently dependent on the X-Y relation. A male hybrid possessing a Rabaul X and a New Guinea Y, and vice versa, or a New Guinea X and a Cairns Y is sterile. The only case of an X-Y incompatibility between geographically isolated strains of *Drosophila* has been reported in *D. micromelanica* by Sturtevant and Novitski (1941). However, hybrid sterility has by no means always been conditioned by X-Y relations but is dependent upon X-autosome or Y-autosome complementary factors as demonstrated by Patterson

et al. (1942) in genetical analyses of hybrids in the *D. virilis* complex, and by Mainland (1942) working on the *D. funebris* group. It seems probable that in *D. birchii* a complementary factor (or factors) in sex chromosome complements may be involved in causing hybrid sterility. Whether or not the sterility involves interactions between the sex-chromosomes and autosomes remains unknown, and it will not be possible to determine its genetic nature until chromosome marker stocks are available.

The chief mechanism that maintains reproductive isolation of the geographic populations under natural conditions is sexual isolation, which prevents the wastage of reproductive effort and consequently results in limiting or preventing the appearance of hybrids. Using the male-choice technique, the results of the present study indicate that the Rabaul strain consistently shows a high index of isolation from other strains. The situation here resembles that of the well-studied *D. rubida*, another Australasian species, as demonstrated by Mather (1964) who has shown that the Rabaul race is highly sexually isolated from the other races.

The situation in *D. birchii* may compare equally well with the extensively studied *D. paulistorum* complex. At least six races in this species have been established on the basis of reproductive isolation which in fact is strong enough to enable them to coexist in some areas without gene exchange (Dobzhansky and Spassky, 1959; Malogolowkin et al., 1965). *D. paulistorum* is regarded as a superspecies of which there exist the so-called transitional race and transitional strains that are capable of producing fertile hybrids with their own as well as with other races (Dobzhansky et al., 1964). In *D. birchii*, the most prominent feature among the isolating mechanisms is geographic isolation, which effectively prevents gene flow from one population to another under natural conditions. As may be seen,

Rabaul, Cairns, and Papua-New Guinea are widely separated from one another by water barriers. Such geographic isolation subsequently permits every opportunity for the isolated populations to develop their own novel systems of coadapted gene complexes in adapting themselves to certain environmental conditions. This assumption appears to have become reality, particularly as observed in the Rabaul and Cairns populations, since each of these exhibits its own unique gene arrangement and inversion polymorphs (Baimai, in prep.). The cytological evidence is thus in harmony with results disclosed in this sexual isolation study. Hence the evidence from the cytogenetic studies has led to the hypothesis that the Australasian species, *D. birchii*, is subdivided into four distinct cytological or geographical races, namely: Rabaul, Cairns, New Guinea, and Daru (transitional form).

It is not difficult to envisage the situation in *D. birchii* coming to resemble that in *D. paulistorum*, if the New Britain populations which presumably belonged to the Rabaul group migrated to the territory of Papua-New Guinea and they coexisted without effective gene exchange. This would mean that the Rabaul group is on its way to speciation and could eventually emerge as a genetically closed system, attaining full species status. On the other hand, the Cairns group appears to have undergone less reproductive isolation from the New Guinea populations than the Rabaul group. Subsequently the expanding of the population into the territory of Papua-New Guinea would bring about the breakdown of reproductive isolation. Furthermore, the existence of the transitional form at Daru would undoubtedly open a channel for gene exchange between the allopatric populations of Cairns and Papua-New Guinea. At our time level, nevertheless, the incipient speciation process in *D. birchii* has not gone very far and consequently offers a probable opportunity to witness evolution in action.

## SUMMARY

The present data on sexual isolation between strains of *D. birchii* of different geographic origins support the view of Ayala (1965) that this Australasian species consists of reproductively isolated subdivisions.

Male hybrids are sterile when either parent is from the Rabaul strain or when the male parent is from Cairns, except when the cross involves the Daru strain, which produces fertile hybrids of both sexes with the Cairns and Papua-New Guinea strains—suggesting the existence of a transitional form at Daru.

High degrees of mating preferences were consistently observed between the Rabaul strain characterized by  $X_4Y_2$  sex chromosomes and the Papua-New Guinea strains with  $X_1Y_1$  sex chromosomes, while considerably lower isolation indices were observed among the strains from Cairns (also characterized by  $X_4Y_2$  sex chromosomes), Daru (characterized by  $X_1Y_3$  sex chromosomes) and Papua-New Guinea.

The geographic isolation is an effective barrier to interbreeding in *D. birchii*. However, the combination of some sexual preferences, hybrid sterility, and differences regarding X- and Y-chromosome types as well as inversion polymorphs suggests an additional effective barrier to interbreeding in nature if the populations were to become sympatric in some areas. The genetic basis of the reproductive isolating mechanisms is less clear.

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