

Evidence for Incipient Sexual Isolation within *Drosophila ananassae*

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Punita Nanda and Bashisth Narayan Singh (2011) Evidence for incipient sexual isolation within *Drosophila ananassae*. *Zoological Studies* 50(5): 577-587. Intraspecific sexual isolation was studied by employing 20 mass culture laboratory stocks of *Drosophila ananassae* established from naturally impregnated females collected from different localities in India using a male-choice technique. In total, 190 crosses involving 20 strains were carried out in which each strain was reciprocally tested with other strains. The results showed that in 54 of 190 crosses, there was a preference for homogamic matings, and differences between homogamic and heterogamic matings were statistically significant in one of the reciprocal crosses; this provides evidence for asymmetry in sexual isolation, while three of 190 crosses showed symmetrical sexual isolation in which positive assortative mating was observed in both reciprocal crosses. In 133 of 190 crosses, there was no sexual isolation. These findings provide evidence for instability of mate recognition leading to behavioral isolation within *D. ananassae*. These results are discussed in light of results reported in other species of *Drosophila*.
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Key words: *Drosophila ananassae*, Genetic divergence, Models of asymmetry, Natural populations, Sexual isolation.

One of the longest running debates in evolutionary biology is over the relative importance of different ecological and demographic factors in causing speciation. Reproductive isolation, responsible for speciation, is likely to involve complex, coevolving, polygenic traits (Templeton 1996). Because of historical reliance on the biological species concept (Mayr 1942, Coyne and Orr 2004), a preliminary step in investigating the degree of reproductive isolation between populations often involves estimating sexual isolation. This can provide a general indication of the strength of premating isolation among populations, and perhaps inferences about a species' status, but does not necessarily inform us of about how premating isolation evolved.

The biological species concept defines species as "groups of interbreeding natural populations that are reproductively isolated

from other such groups" (Mayr 1942). Under this concept, new species evolve by acquiring reproductive isolation between populations. The enumeration, classification, and analysis of reproductively isolating mechanisms are central tasks for studying speciation, especially for many biologists who adhere to the biological species concept (Dobzhansky 1935, Mayr 1942). The genetic architecture underlying reproductively isolating traits may have substantial impacts on the likelihood and pace of speciation. Dobzhansky (1937) coined the term 'isolating mechanisms' for genetically conditioned barriers that prevent gene flow between Mendelian populations. These mechanisms are characterized as premating, postmating-prezygotic, and postzygotic barriers (Coyne and Orr 2004). Reproductive isolation is an important mechanism that ensures the uniqueness and integrity of a species' gene pool.

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Different theories were proposed to explain how reproductively isolating mechanisms originate between populations. According to Muller (1942), reproductive barriers appear as a side effect of genetic divergence because populations adapted to different environments acquire genetic differences which lead to reproductive isolation. Dobzhansky (1940) gave more importance to natural selection acting on appropriate genetic variation when allopatric populations that were incipiently isolated become sympatric. Carson (1971) emphasized the role of genetic drift through the founder effect in the origin of reproductive isolation which serves as a barrier to gene flow. Carson's (1975) "flush-crash" speciation theory was supported by the work of Powell (1978), who observed that strong assortative mating developed between cage populations of *Drosophila pseudoobscura* which had passed through successive bottlenecks in population size.

Sexual or ethological isolation is a premating barrier to gene exchange in which opposite sexes of different populations fail to mate due to behavioral incompatibility. There was some progress in the last decade in understanding the evolution of reproductive isolation, but this subject still remains a major challenge (Templeton 1996, Coyne and Orr 2004). Coyne and Orr (1989) investigated premating and postmating isolation in several *Drosophila* species and concluded that among sympatric species pairs, premating isolation arises more rapidly than postmating isolation. The evolution of premating isolation caused by divergent mating signals and preferences appears to be an important component of speciation in many taxa (Coyne and Orr 2004).

Mating signals may be reciprocally exchanged between the sexes, allowing for mate recognition. Divergence of such mating signals and recognition systems can lead to speciation (Coyne and Orr 2004). Behavioral traits that are involved in a wide range of mating cues and preferences fall under the generalized category of sex traits. Such characters, often species- and/or genus-specific can greatly diverge between species. Mate recognition systems consist of secondary sexual characteristics in 1 sex (usually males) associated with a preference for the trait(s) in the other sex (usually females) (Servedio and Saetre 2003). These systems are considered important species attributes which act as cohesive forces (Paterson 1980). Paterson (1978) argued that the evolution of a specific mate-recognition system, composed of multiple "coadapted stages", is essential to

intersexual signaling and is likely to be maintained by strong stabilizing selection.

In the genus *Drosophila*, the phenomenon of sexual isolation (both intraspecific and interspecific) has been extensively studied and found to be widespread (Ehrman and Parsons 1980, Henderson and Lambert 1982, Spieth and Ringo 1983, Hollocher et al. 1997, Singh 1997, Ting et al. 2001, Haerty et al. 2005, Castrezana and Markow 2008, Schug et al. 2008, Yukilevich and True 2008a). There are certain studies in *Drosophila* which show that sexual isolation between closely related species is stronger between sympatric rather than allopatric populations (Coyne and Orr 1989). Such results were found in the semi-species group of *D. paulistorum* (Ehrman 1965).

Drosophila ananassae belongs to the *ananassae* species complex of the *ananassae*-subgroup of the *melanogaster* species group. It is a cosmopolitan and domestic species. Sexual isolation, maintained by strong mating preferences was reported in light and dark forms of *D. ananassae* in laboratory stocks (Futch 1966). These forms were found to be sibling species: *D. ananassae* and *D. pallidosa* (Futch 1973, Doi et al. 2001, Sawamura et al. 2006, Vishalakshi and Singh 2006). In spite of their sympatric distribution, post-mating reproductive barriers such as hybrid sterility or hybrid inviability do not exist between them (Futch 1966, Bock and Wheeler 1972). Analyses of Y-chromosomal and mitochondrial haplotypes, shared chromosomal arrangements, premating isolation, and hybrid male sterility suggested that these taxa represent a recent evolutionary radiation and have experienced substantial gene flow (Matsuda et al. 2009). Genes controlling sexual behavior are likely to control species-specific differences in courtship and are involved in reproductive isolation of closely related species of *Drosophila* (Sisodia and Singh 2005).

In an attempt to gain insights into the process of developing reproductive isolation, 20 mass culture laboratory stocks of *D. ananassae* established using flies collected from ecologically different localities of India with different collection times were studied. These stocks were reciprocally tested against each other that make 190 crosses in which implications of asymmetrical premating sexual isolation in *D. ananassae* on the speciation history of this species are discussed.

MATERIALS AND METHODS

Drosophila stocks

Twenty mass culture stocks of *D. ananassae* established from females collected from different eco-geographical localities (Table 1) in India (Fig. 1) were used. These stocks were derived from naturally impregnated females which had been kept for varying number of generations under laboratory conditions. All stocks were maintained on simple yeast-agar culture medium by transferring about 50 flies (with equal numbers of males and females) to fresh food bottles in each generation under laboratory conditions at approximately 24°C. Virgin flies were collected under light ether anesthesia within a few hours (6-7 h) of eclosion. Females and males were stored separately in vials with fresh food in batches of 15 to avoid bias resulting from density (Knoppien 1985).

Marking procedure

Virgin females and males collected from different populations were aged for 7 d and then examined under anesthesia for any obvious morphological distortions that might have taken

place during the period of aging. Only normal flies were used. In order to identify flies of different strains, the distal part of the right wing of flies of 1 strain was clipped 1 d before the experiment. A male choice-technique was used in which females were marked by wing-clipping in order to distinguish them from flies of different strains.

Mate-choice experiments

By employing the male-choice technique, males of 1 type were kept with females of both types, i.e., 15 males of 1 type with 15 females of each of the 2 types, and patterns of matings were recorded. The total number of flies in each trial was 45, the sex ratio was 1 male: 2 females, and 5 replicates were run for each experimental set. Before making the cross, the effect of marking was tested, and the results indicated that marking had no effect on either the performance of flies or the outcome of the sexual isolation tests (Som and Singh 1998, Nanda and Singh 2008). Flies were introduced into an Elens-Wattiaux (1964) mating chamber without etherization, with females being introduced first. Matings were observed for 60 min. When a pair commenced mating, it was aspirated out and kept in separate empty vials (Spiess 1968). Later, mating types were identified using

Table 1. Details of the different strains of *D. ananassae* used in the present study

| Strains | Place of origin | No. of founding females | Time of collection |
|---------|----------------------------|-------------------------|--------------------|
| BP | Birlapur, West Bengal | 100 | 1985 |
| QL | Quilon, Kerala | * | 1985 |
| BR | Baripada, Orissa | 22 | 1987 |
| PC | Pondicherry, Tamil Nadu | 26 | 1999 |
| IT | Itarsi, Madhya Pradesh | 3 | 2000 |
| MY | Mysore, Karnataka | 6 | 2000 |
| RM | Ramna, Uttar Pradesh | * | 2000 |
| PJ | Panjim, Goa | 3 | 2001 |
| SK | Shaktinagar, Uttar Pradesh | 8 | 2002 |
| CV | Chavara, Kerala | 24 | 2003 |
| DW | Dwarka, Gujarat | 90 | 2005 |
| LK | Lucknow, Uttar Pradesh | 48 | 2005 |
| DM | Deemapur, Nagaland | 211 | 2006 |
| GT | Gangtok, Sikkim | 34 | 2006 |
| JU | Jammu, Jammu and Kashmir | 130 | 2006 |
| KR | Kanniyakumari, Tamil Nadu | 56 | 2006 |
| MU | Mumbai, Maharashtra | 99 | 2006 |
| SI | Shirdi, Maharashtra | 103 | 2006 |
| DL | New Delhi | 50 | 2007 |
| VN | Varanasi, Uttar Pradesh | 5 | 2007 |

Results of male-choice experiments among different laboratory strains of *D. ananassae*.

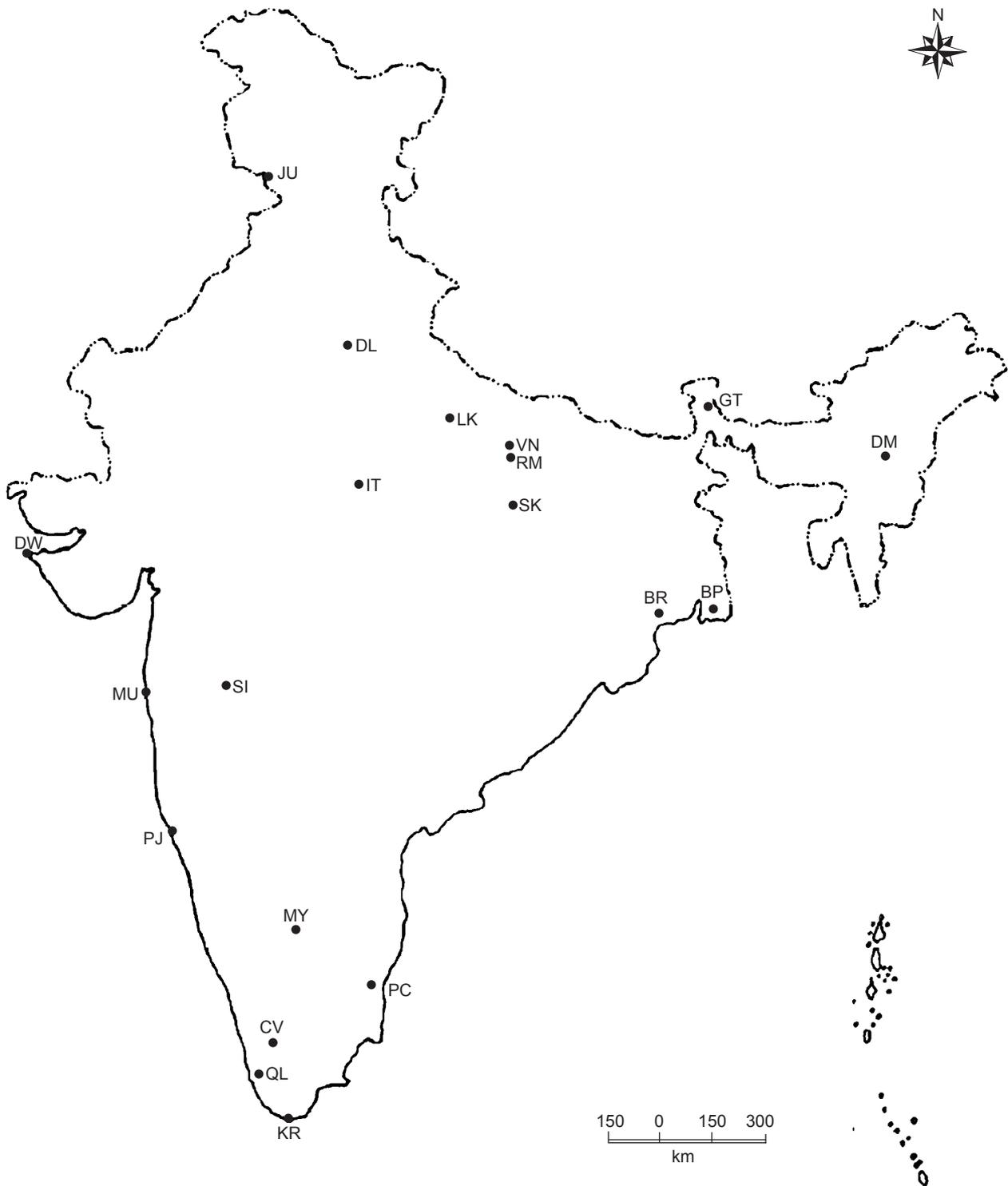


Fig. 1. Map of India showing the localities from where the flies of *Drosophila ananassae* were collected. BP, Birlapur; QL, Quilon; BR, Baripada; PC, Pondicherry; IT, Itarsi; MY, Mysore; RM, Ramna; PJ, Panjim; SK, Shaktinagar; CV, Chavara; DW, Dwarka; LK, Lucknow; DM, Deemapur; GT, Gangtok; JU, Jammu; KR, Kanniyakumari; MU, Mumbai; SI, Shirdi; DL, New Delhi; and VN, Varanasi.

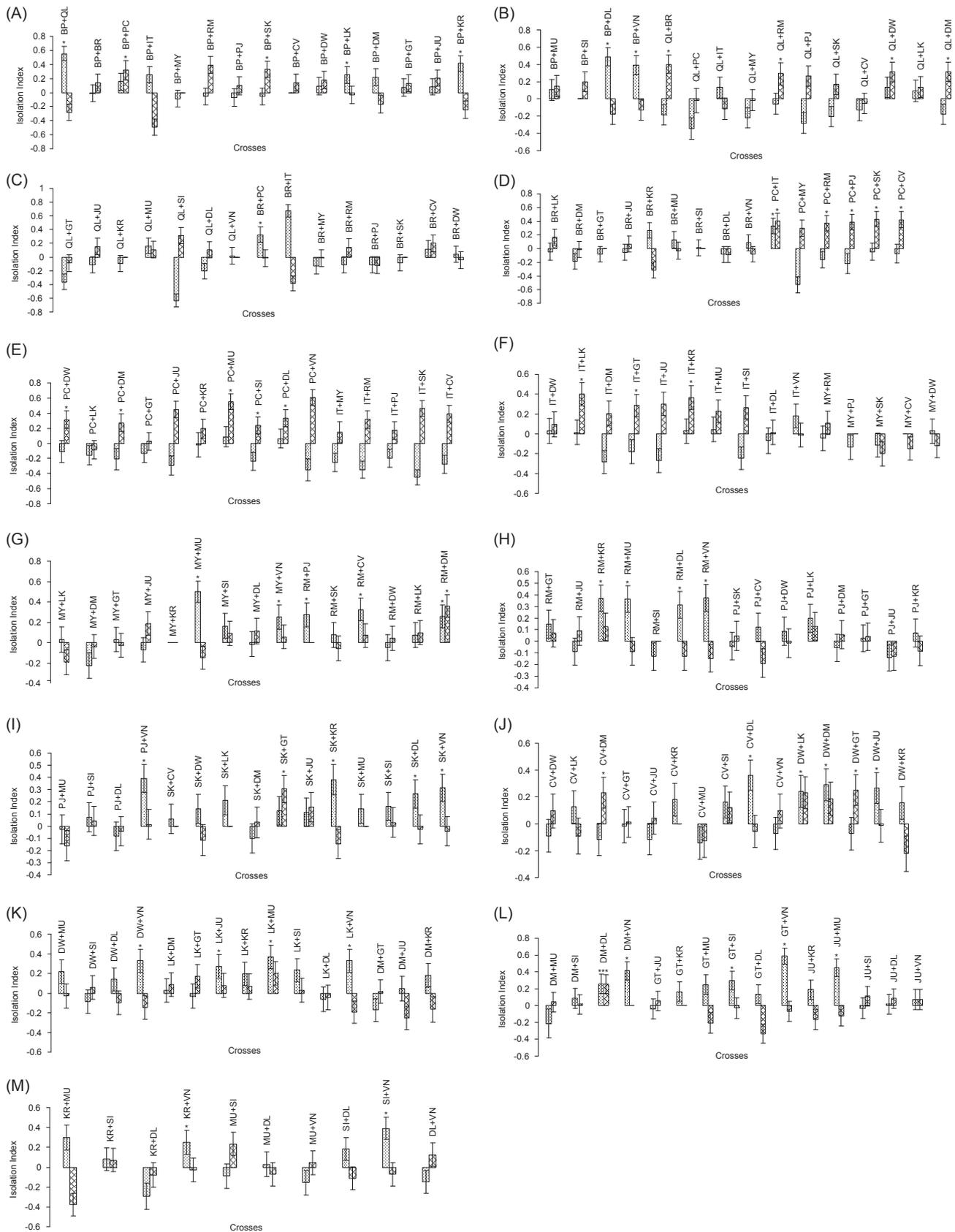


Fig. 2. (A-M) Isolation indices in different crosses involving laboratory stocks of *Drosophila ananassae*. * $p < 0.05$

females discriminated against males of 11 strains (RM, PJ, SK, DW, LK, DM, GT, KR, SI, BP, and MY). In crosses PC × IT, DM × DL, and RM × DM, evidence of positive assortative mating was found in reciprocal crosses that made chi square values significant and isolation indices significantly > 0, providing evidence for symmetrical isolation. From these results, it is clear that the pattern and degree of sexual isolation vary in different crosses. The maximum degree of sexual isolation occurred between the GT and VN strains with GT males, in which one-sided mating preference was observed (0.589), and in reciprocal cross mating was random. The present results indicate that laboratory strains of *D. ananassae* tested during the present study showed a considerable degree of incipient sexual isolation.

DISCUSSION

The present results of intraspecific sexual isolation tests provide evidence that laboratory strains of *D. ananassae* have developed behavioral reproductive isolation as a result of positive assortative mating. These strains have accumulated considerable amounts of genetic divergence that affect the mate-recognition system, and the strains showed incipient isolation. It is known that natural and laboratory populations of *D. ananassae* show a considerable degree of genetic divergence at the level of chromosomal polymorphism, display population sub-structuring, and exist as semi-isolated populations (Singh and Singh 2007 2008 2010).

The results obtained for *D. ananassae* clearly show that sexual isolation has evolved as a by-product of ecological divergence in its natural populations and due to genetic drift in laboratory populations that have been maintained for many generations in the laboratory. Dobzhansky (1940) postulated that ethological isolation evolves as an ad hoc product of natural selection because populations adapted to different environments develop different co-adapted gene complexes. Recently collected strains showing isolation demonstrates that natural selection is responsible for causing isolation, and strains that have spent varying number of generations under laboratory conditions showing isolation indicates the action of random genetic drift causing sexual isolation. Muller (1942) suggested that reproductive barriers might arise as a consequence of genetic drift or as an accidental by-product of genetic divergence

as organisms adapt to different environments. Therefore, for origin and maintenance of races and species, isolation is an indispensable factor, and its importance has been recognized for a long time (Singh 2010).

The formation of premating isolating barriers among recently diverged populations is often an early step in the process of speciation (Dobzhansky 1937, Coyne and Orr 2004). Establishing the causes for different patterns of mate choice among populations usually requires analysis of multiple causes including mate preference for conspecifics or species recognition systems (Paterson 1993, Gerhardt and Huber 2002), natural selection via reinforcement (Noor 1999, Servedio and Noor 2003), and sensory bias (Ryan 1998, Boughman 2002).

Henderson and Lambert (1982) suggested that there is considerable stability in mate-recognition systems of widely separated populations of *D. melanogaster* despite the substantial genetical and morphological differentiation that exists throughout the cosmopolitan distribution of this species. In *D. ananassae*, mate discrimination varies considerably throughout the species range, being higher among populations outside the ancestral Indonesian range and the highest in the South Pacific (Schug et al. 2008). Results suggest that colonization and genetic differentiation affect the evolutionary origin of mate discrimination (Schug et al. 2008). The degree of sexual isolation is stronger in isofemale lines than in natural populations of *D. ananassae* and may involve genetic bottlenecks (Singh and Chatterjee 1985).

Sexual isolation is a consequence of genetic changes that have accumulated in populations during the speciation process that have resulted in species discrimination due to modifications in male and female mating behaviors (Tomaru et al. 1995) and patterns of pheromonal cuticular composition (Coyne and Orr 2004, Yukilevich and True 2008b). Pheromonal differences between species can influence sexual isolation in many animals, and in some cases, a single locus can cause large functional changes in pheromonal mating signals (Gleason et al. 2009). *Drosophila* cuticular hydrocarbons (CHCs) function as pheromones and consequently affect mate-recognition systems (Gleason et al. 2005). Two major CHCs in females affect mating discrimination between *D. simulans* and *D. sechellia*. Quantitative trait loci (QTLs) were identified on the X and 3rd chromosomes, and a few candidate genes were potentially

implicated (Gleason et al. 2005 2009).

Dobzhansky (1937) and Muller (1942) suggested that divergence among populations in allopatry can lead to sexual isolation arising as a pleiotropic by-product of genetic drift or local adaptation. If behavioral divergence results in reduced gene flow, increasing genetic differentiation among populations may help preserve incipient reproductive isolation, and if selection is strong enough, reproductive isolation can persist in sympatry despite a low level of interpopulational gene flow (Bush 1969). Most empirical studies of reproductive isolation, especially of sexual isolation, confirmed the polygenic effects (Hollocher et al. 1997, Ritchie and Phillips 1998, Ting et al. 2001). However, a few large-effect genes were identified for both pre-mating (Greenberg et al. 2003) and post-mating isolation (Barbash et al. 2003, Presgraves et al. 2003).

In *D. ananassae*, heterosis was found to be associated with alpha inversion and the male mating ability, as heterokaryotypic males had superior mating activity compared to homokaryotypic ones (Singh and Chatterjee 1986). Thus, inversion polymorphism may have a partial behavioral basis, and males are more often subject to intrasexual selection than females (Singh and Chatterjee 1986). Inversions may facilitate the accumulation of alleles that contribute to reproductive isolation between populations connected by gene flow. This is particularly true for Bateson-Dobzhansky-Muller (BDM) incompatibilities; otherwise, the ancestral, compatible genotype would be favored (Noor et al. 2001). Behavioral isolation was found between 2 karyotypically different homozygous strains of *D. ananassae* derived from the same geographic location, which shows that chromosome arrangements can affect mate-recognition system in *D. ananassae* (Nanda and Singh 2011a). It was also demonstrated that behavioral isolation can originate due to founder effects in *D. ananassae* (Nanda and Singh 2011b), which supports the founder principle of Mayr (1942). Servedio and Saetre (2003) found that linkages between genes affected prezygotic and postzygotic isolation leading to a positive feedback loop in which both were strengthened. Those investigators also found that genes causing hybrid incompatibility hitchhike along with those improving pre-mating isolation, leading to stronger hybrid incompatibility, and there may be coevolution of prezygotic and postzygotic barriers that are enhanced by sex

linkages of genes affecting mate recognition and hybrid viability (Servedio and Saetre 2003).

Doi et al. (2001) identified possible loci that control female discrimination in *D. ananassae* and *D. pallidosa* which were mapped on distinct positions near the *Delta* locus in the middle of 2L. Yamada et al. (2002a b) in their extensive studies of sexual isolation between 2 sibling and sympatric species, *D. ananassae* and *D. pallidosa*, recorded and analyzed male courtship songs and observed species specificity in the courtship song parameters which was the basis of sexual isolation between these species. Etges et al. (2007) studied mating success and courtship song differences among divergent populations of *D. mojavensis* in a QTL analysis and found that the genetics of incipient speciation in *D. mojavensis* depends on cactus-specific expression of traits associated with courtship behavior and sexual isolation. They suggested that these parameters play a role in mate recognition and enforce sexual isolation. Multiple regression analyses using interspecific mosaic genome lines of *D. ananassae* and *D. pallidosa* indicated highly significant effects on 2L of female mating willingness with *D. ananassae* males and on XL, 2L, and 3R for mating with *D. pallidosa* males (Sawamura et al. 2008).

Geographically distant populations are more likely to experience both divergent ecological selective conditions and a reduction in homogenizing gene flow, which is likely to be one of the greatest impediments to the evolution of isolating mechanisms (Dobzhansky 1937, Mayr 1942, Coyne and Orr 2004). *D. ananassae* is a cosmopolitan and domestic species. All domestic species are characterized by a high incidence of interpopulation migration because of their association with man. Futch (1966) pointed out that populations of *D. ananassae* separated by oceans and mountains may experience some gene exchange because of its close association with man. Dobzhansky and Dreyfus (1943) suggested that this species has depended on man for its present widespread distribution.

Recently, there was a resurgence of interest in the ecological causes of speciation and the ways in which natural selection may play a primary role in the evolution of reproductive isolation (Schluter 2001). New theoretical and empirical approaches have shown how interspecific interactions and differences among environments (causes of natural selection) can interact with the genetics of phenotypic traits to produce the evolution of reproductive isolation and the splitting of lineages

into separate species (Schluter 2001, Via 2001). The interplay between natural selection and genetic variability is the essence of the approach to the study of evolution known as ecological genetics.

Some studies indicated that there was no correlation between asymmetrical mate choice and polarity of mating behavior (Wasserman and Koepfer 1980, Markow 1981, Moodie 1982, Koepfer 1991), except that Kaneshiro (1976) and Watanabe and Kawanishi (1979) proposed the opposite. There are 2 hypotheses with respect to asymmetrical reproductive isolation. One states that ancestral females prefer to mate with conspecific males, but derived females do not (Kaneshiro 1976) as observed in Hawaiian *Drosophila*; the other proposes that derived females prefer to mate with conspecific males (Watanabe and Kawanishi 1979). Watanabe and Kawanishi (1979) found that in species evolved with overlapping ranges, derived females prefer conspecific males, but ancestral females do not. The former might have involved loss of some courtship elements through genetic drift or adaptations to different environments (Kaneshiro 1976), which explains the rejection of a derived male by an ancestral female. In the latter case without an effective geographical barrier, if derived females reject original males, then the derived population might have a higher chance of differentiating from the original population. This might be related to Watanabe and Kawanishi's model. These derived males may have evolved new male performances instead of having lost some courtship elements. Chang and Tai (2007) studied asymmetrical reproductive isolation between *D. albomicans* and *D. nasuta*, and implications of this asymmetrical premating sexual behavior in *D. albomicans* on the speciation history of this species were discussed.

These findings provide evidence that there is incipient sexual isolation among mass culture laboratory stocks derived from natural populations of *D. ananassae*, which shows that there is instability of the mate-recognition system. Further, these findings also provide evidence that models proposed to predict the direction of evolution based on asymmetrical isolation do not have general applicability in the genus *Drosophila* (see Markow 1981).

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REFERENCES

- Barbash DA, DF Siino, AM Tarone, J Roote. 2003. A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. Proc. Natl. Acad. Sci. USA. **100**: 5302-5307.
- Bock IR, MR Wheeler. 1972. The *Drosophila melanogaster* species group. Univ. TX Publ. **7213**: 1-102.
- Boughman JW. 2002. How sensory drive can promote speciation. Trends Ecol. Evol. **17**: 571-577.
- Bush GL. 1969. Sympatric host race formation and specialization in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution **23**: 237-251.
- Carson HL. 1971. Speciation and founder principle. Stadler Genet. Symp. **3**: 51-70.
- Carson HL. 1975. Genetics of speciation. Am. Nat. **109**: 83-92.
- Castrezana SJ, TA Markow. 2008. Sexual isolation and mating propensity among allopatric *Drosophila mettleri* populations. Behav. Genet. **38**: 437-445.
- Chang H, Y Tai. 2007. Asymmetrical reproductive isolation between *Drosophila albomicans* and *D. nasuta*. Zool. Stud. **46**: 638-646.
- Coyne JA, HA Orr. 1989. Patterns of speciation in *Drosophila*. Evolution **43**: 362-381.
- Coyne JA, HA Orr. 2004. Speciation. Sunderland, MA: Sinauer and Associates.
- Dobzhansky T. 1935. A critique of the species concept in biology. Phil. Sci. **2**: 344-345.
- Dobzhansky T. 1937. Genetics and the origin of species. New York: Columbia Univ. Press.
- Dobzhansky T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. **74**: 312-321.
- Dobzhansky T, A Dreyfus. 1943. Chromosomal aberrations in Brazilian *Drosophila ananassae*. Proc. Natl. Acad. Sci. USA **29**: 301-305.
- Doi M, M Matsuda, M Tomaru, H Matsubayashi, Y Oguma. 2001. A locus for female discrimination behaviour causing sexual isolation in *Drosophila*. Proc. Natl. Acad. Sci. USA **98**: 6714-6719.
- Ehrman L. 1965. Direct observations of sexual isolation between allopatric and sympatric strains of different *Drosophila paulistorum* races. Evolution **19**: 459-464.
- Ehrman L, PA Parsons. 1980. Sexual isolation among widely distributed populations of *Drosophila immigrans*. Behav. Genet. **10**: 401-407.
- Elens AA, JM Wattiaux. 1964. Direct observation of sexual isolation. Drosoph. Inf. Serv. **39**: 118-119.
- Etges WJ, CC Oliveira, E Gragg, D Ortiz-Barrientos, MAF Noor, MG Ritchie. 2007. Genetics of incipient speciation in *Drosophila mojavensis*. I. Male courtship song, mating success, and genotype x environment interactions. Evolution **61**: 1106-1119.
- Futch DG. 1966. A study of speciation in South Pacific populations of *Drosophila ananassae*. Univ. TX Publ. **6615**: 79-120.
- Futch DG. 1973. On the ethological differentiation of *Drosophila ananassae* and *Drosophila pallidosa* in

- Samoa. *Evolution* **27**: 299-312.
- Gerhardt HC, F Huber. 2002. *Acoustic communications in insects and anurans*. Chicago, IL: Univ. of Chicago Press.
- Gleason JM, JM Jallon, JD Rouault, MG Ritchie. 2005. Quantitative trait loci for cuticular hydrocarbons associated with sexual isolation between *Drosophila simulans* and *D. sechellia*. *Genetics* **171**: 1789-1798.
- Gleason JM, RA James, C Wicker-Thomas, MG Ritchie. 2009. Identification of quantitative trait loci function through analysis of multiple cuticular hydrocarbons differing between *Drosophila simulans* and *Drosophila sechellia* females. *Heredity* **103**: 416-424.
- Greenberg AJ, JR Moran, JA Coyne, CI Wu. 2003. Ecological adaptation during incipient speciation revealed by precise gene replacement. *Science* **302**: 1754-1757.
- Haerty W, M Lesbats, P Capy. 2005. Pre-reproductive isolation as a consequence of allopatric differentiation between populations of *Drosophila melanogaster*. *Mol. Ecol.* **14**: 3801-3807.
- Henderson NR, DM Lambert. 1982. No significant deviation from random mating of worldwide populations of *Drosophila melanogaster*. *Nature* **300**: 437-440.
- Hollocher H, CT Ting, ML Wu, CI Wu. 1997. Incipient speciation by sexual isolation in *Drosophila melanogaster*: the genetics of the Zimbabwe race. *Genetics* **147**: 1191-1211.
- Kaneshiro KY. 1976. Ethological isolation and phylogeny in planitibia subgroup of Hawaiian *Drosophila*. *Evolution* **30**: 740-745.
- Knopp P. 1985. The number of males stored per vial: a possible source of bias in rare male experiments. *Drosoph. Inf. Serv.* **61**: 101.
- Koepfer HR. 1991. Asymmetrical mating patterns between geographic strains of *Drosophila mercatorum*: a test of the Kaneshiro hypothesis. *Evolution* **45**: 455-458.
- Markow TA. 1981. Mating preferences are not predictive of the direction of evolution in *Drosophila*. *Science* **213**: 1405-1407.
- Matsuda M, CS Ng, M Doi, A Kopp, YN Tobar. 2009. Evolution in the *Drosophila ananassae* species subgroup. *Fly* **3**: 157-169.
- Mayr E. 1942. *Systematics and the origin of species*. New York: Columbia Univ. Press.
- Moodie GEE. 1982. Why asymmetric mating preference may not show the direction of evolution. *Evolution* **36**: 1096-1097.
- Muller HJ. 1942. Isolating mechanisms, evolution and temperature. *Biol. Symp.* **6**: 71-125.
- Nanda P, BN Singh. 2008. No effect of marking procedures and choice situations on the pattern of matings in *Drosophila ananassae*. *Drosoph. Inf. Serv.* **91**: 10-13.
- Nanda P, BN Singh. 2011a. Effect of chromosome arrangements on mate recognition system leading to behavioral isolation in *Drosophila ananassae*. *Genetica* **139**: 273-279.
- Nanda P, BN Singh. 2011b. Origin of sexual isolation in *Drosophila ananassae* due to founder effects. *Genetica* **139**: 779-787.
- Noor MAF. 1999. Reinforcement and other consequences of sympatry. *Heredity* **83**: 503-508.
- Noor MAF, KL Grams, LA Bertucci, J Reiland. 2001. Chromosomal inversions and the reproductive isolation of species. *Proc. Natl. Acad. Sci. USA* **98**: 12084-12088.
- Paterson HE. 1978. More evidence against speciation by reinforcement. *S. Afr. J. Sci.* **74**: 369-371.
- Paterson HE. 1980. A comment on 'mate recognition systems'. *Evolution* **34**: 330-331.
- Paterson HE. 1993. *Evolution and the recognition concept of species: collected writings*. Baltimore, MD: John Hopkins Univ. Press.
- Powell JR. 1978. The founder-flush speciation theory: an experimental approach. *Evolution* **32**: 465-474.
- Presgraves DC, L Balagopalan, SM Abmayr, HA Orr. 2003. Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. *Nature* **423**: 715-719.
- Ritchie MG, SDF Philips. 1998. The genetics of sexual isolation. In DJ Howard, SH Berlocher, eds. *Endless forms: species and speciation*. New York: Oxford Univ. Press, pp. 291-308.
- Ryan MJ. 1998. Sexual selection, receiver biases, and the evolution of sex differences. *Science* **281**: 1999-2003.
- Sawamura K, Y Tomimura, H Sato, H Yamada, M Matsuda, Y Oguma. 2006. Establishing interspecific mosaic genome lines between *Drosophila ananassae* and *D. pallidosa* by means of parthenogenesis. *Genet. Res.* **88**: 1-11.
- Sawamura K, H Zhi, K Setoguchi, H Yamada, T Miyo, M Matsuda, Y Oguma. 2008. Genetic analysis of female mating recognition between *Drosophila ananassae* and *Drosophila pallidosa*: application of interspecific mosaic genome lines. *Genetica* **133**: 179-185.
- Schluter D. 2001. Ecology and the origin of species. *Trends Ecol. Evol.* **16**: 372-380.
- Schug MD, JF Baines, K Amada, S Mohanty, A Das, S Grath et al. 2008. Evolution of mating isolation between populations of *Drosophila ananassae*. *Mol. Ecol.* **17**: 2706-2721.
- Servedio MR, MAF Noor. 2003. The role of reinforcement in speciation: theory and data. *Ann. Rev. Ecol. Evol. Syst.* **34**: 339-364.
- Servedio MR, GP Saetre. 2003. Speciation as a positive feedback loop between postzygotic and prezygotic barriers to gene flow. *Proc R Soc Lond B* **270**: 1473-1479.
- Singh BN. 1997. Mode of mating preference and the direction of evolution in *Drosophila*. *Ind. J. Exp. Biol.* **35**: 111-119.
- Singh BN. 2010. The origin of reproductive isolating mechanisms is an important event in the process of speciation: evidences from *Drosophila*. In VP Sharma, ed. *Nature at work: ongoing saga of evolution*. New Delhi, India: Springer Private Limited, pp. 159-173.
- Singh BN, S Chatterjee. 1985. Symmetrical and asymmetrical sexual isolation among laboratory strains of *Drosophila ananassae*. *Can. J. Genet. Cytol.* **27**: 405-409.
- Singh BN, S Chatterjee. 1986. Mating ability of homo- and heterokaryotypes of *Drosophila ananassae* from natural populations. *Heredity* **57**: 75-78.
- Singh P, BN Singh. 2007. Population genetics of *Drosophila ananassae*: genetic differentiation among Indian natural populations at the level of inversion polymorphism. *Genet. Res.* **89**: 191-199.
- Singh P, BN Singh. 2008. Population genetics of *Drosophila ananassae*: variation in the degree of genetic divergence in populations transferred to laboratory conditions. *Zool Stud.* **47**: 704-712.
- Singh P, BN Singh. 2010. Population genetics of *Drosophila ananassae*: evidence for population sub-structuring at the level of inversion polymorphism in Indian natural

- populations. *Int. J. Biol.* **2**: 19-28.
- Sisodia S, BN Singh. 2005. Genetics of sexual behavior in *Drosophila*. *Ind. J. Exp. Biol.* **43**: 575-595.
- Som A, BN Singh. 1998. No effect of marking the flies either by nail polish on scutellum or by wing clipping on mating success in *Drosophila ananassae*. *Drosoph. Inf. Serv.* **81**: 202-203.
- Speith HT, JM Ringo. 1983. Mating behavior and sexual isolation in *Drosophila*. In MA Ashburner, HL Carson, JN Thompson Jr, eds. *The genetics and biology of Drosophila*. Vol. 3c. New York: Academic Press, pp. 223-284.
- Spieß EB. 1968. Low frequency advantage in mating of *Drosophila pseudoobscura* karyotypes. *Am. Nat.* **102**: 363-379.
- Stalker HD. 1942. Sexual isolation in the species complex *Drosophila virilis*. *Genetics* **27**: 238-257.
- Templeton AR. 1996. Experimental evidence for the genetic-transilience model of speciation. *Evolution* **50**: 909-915.
- Ting CT, A Takahashi, CI Wu. 2001. Incipient speciation by sexual isolation in *Drosophila*: concurrent evolution at multiple loci. *Proc. Natl. Acad. Sci. USA* **98**: 6709-6713.
- Tomaru M, H Matsubayashi, Y Oguma. 1995. Heterospecific inter-pulse intervals of courtship songs elicit female rejection in *Drosophila biauaria*. *Anim. Behav.* **50**: 905-914.
- Via S. 2001. Sympatric speciation in animals: the ugly duckling grows up. *Trends Ecol. Evol.* **16**: 381-390.
- Vishalakshi C, BN Singh. 2006. Sexual isolation between two sibling species of *Drosophila*: *D. ananassae* and *D. pallidosa*. *Curr. Sci.* **90**: 1003-1006.
- Wasserman M, HR Koepfer. 1980. Does asymmetrical mating preference show the direction of evolution? *Evolution* **34**: 1116-1126.
- Watanabe TK, M Kawanishi. 1979. Mating preference and the direction of evolution in *Drosophila*. *Science* **205**: 906-907.
- Yamada H, M Matsuda, Y Oguma. 2002a. Genetics of sexual isolation based on courtship song between two sympatric species, *Drosophila ananassae* and *D. pallidosa*. *Genetica* **116**: 225-237.
- Yamada H, T Sakai, M Tomaru, M Doi, M Matsuda, Y Oguma. 2002b. Search for species specific mating signal in courtship songs of sympatric sibling species, *Drosophila ananassae* and *D. pallidosa*. *Gene. Genet. Syst.* **77**: 97-106.
- Yukilevich R, JR True. 2008a. Incipient sexual isolation among cosmopolitan *Drosophila melanogaster* populations. *Evolution* **62**: 2112-2121.
- Yukilevich R, JR True. 2008b. African morphology, behavior and pheromones underlie incipient sexual isolation between US and Caribbean *Drosophila melanogaster*. *Evolution* **62**: 2807-2828.
- Zouros E, CJ d'Entremont. 1980. Sexual isolation among populations of *Drosophila mojavensis*: response to pressure from a related species. *Evolution* **34**: 421-430.