Strong reproductive isolation among African species of the *Drosophila montium* subgroup.

Kopp, Artyom. Department of Evolution and Ecology, University of California Davis, Davis CA 95616; akopp@ucdavis.edu

Introduction

The *Drosophila montium* species subgroup is the largest lineage within the *melanogaster* species group, comprising ~90 described species. Although there have been several studies of phylogenetic relationships (Zhang *et al.*, 2003; Yassin *et al.*, 2016; Yang *et al.*, 2012; Da Lage *et al.*, 2007; Miyake and Watada, 2007; Catullo and Oakeshott, 2014), behavior, and reproductive isolation (Kim *et al.*, 1989) in this subgroup, it remains under-studied compared to many other *Drosophila* lineages. At this time, only ~40% of described species are available in live culture.

The *montium* subgroup shows extensive sex-specific variation in the pigmentation of posterior abdominal segments (Yassin *et al.*, 2016; Ohnishi and Watanabe, 1985). In contrast to many other *Drosophila* lineages, female pigmentation in this subgroup evolves more rapidly than male pigmentation (Yassin *et al.*, 2016). Although the females of over 20 different species are polymorphic for light/dark abdominal pigmentation, males of any given species are monomorphic light or monomorphic dark. The only known exception is *D. truncata*, where males as well as females are polymorphic (Ohnishi and Watanabe, 1985). In an effort to map the genetic basis of interspecific variation in male abdominal pigmentation, I have attempted to hybridize closely related African *montium* subgroup species. With the strains I was able to test, these attempts failed.

Materials and Methods

The following species and strains were used in the attempted crosses:

D. nikananu 14028-0601.00 ("nikananu 0")
D. nikananu 14028-0601.01 ("nikananu 1")
D. tsacasi 14028-0701.00
D. bocqueti 14028-0771.00
D. chauvacae 14028-0761.00
D. burlai 14028-0781.00
D. diplacantha 14028-0586.00
D. bakoue Sao Tome

The strain of *D. bakoue* was collected and kindly sent to me by Dr. Jean David. All other strains were obtained from the US *Drosophila* species stock center at the University of California, San Diego.

Crosses were performed between all pairs of species where one parental species had dark males (*D. tsacasi, D. bocqueti, D. chauvacae, D. burlai*, and *D. diplacantha*) and the other had light males (*D. nikananu* and *D. bakoue*) (Table 1). Each cross was performed in both directions. For each reciprocal cross, five mass cultures were set up in vials using at least 20 virgin females and 20 males per vial. Crosses were kept on standard *Drosophila* media at room temperature and ambient light cycle, and transferred to fresh media twice a week until all adults were dead. Vials were inspected regularly for the presence of larvae. All emerging F_1 adults were back-crossed to either the light or the dark parental species in an effort to obtain F_2 progeny. When no F_2 progeny were obtained, a subset of F_1 adults were dissected in insect saline and inspected for the presence of sperm.

Results

Most crosses did not produce any F_1 progeny during the ~2 month life span of the parental adults (Table 1). For these crosses, 5-10 females that were at least 1 month old were dissected per cross, and none were found to carry any sperm, indicating complete pre-mating isolation between the tested strains under these

experimental conditions. The only exception was the cross between *D. tsacasi* and *D. bakoue*, which produced F_1 adults in both directions.

Table 1. Attempted crosses among African montium subgroup species.

	nikananu 0	*nikananu 1*	*bakoue*	*tsacasi*	*bocqueti*	*chauvacae*	*burlai*	*diplacantha*
nikananu 0				-	-	-	-	-
nikananu 1				-	-	-	-	-
bakoue				+	-	-	-	-
tsacasi	-	-	+					
bocqueti	-	-	-					
chauvacae	-	-	-					
burlai	-	-	-					
diplacantha	-	-	-					

Each row indicates the maternal parent species, and each column the paternal parent. "+" indicates that F_1 progeny were obtained, "-" indicates that no F_1 progeny were obtained, and empty cells reflect crosses that were not attempted.

In the cross between *D. bakoue* females and *D. tsacasi* males, several hundred F_1 females and zero males were obtained. Most F_1 females had defective tergite cuticles, suggesting an impairment in the proliferation of abdominal histoblasts. All F_1 females were back-crossed to either *D. tsacasi* or *D. bakoue* males, but did not lay any eggs in either backcross. 10 F_1 females from this cross were dissected, and all were found to have very small degenerate ovaries that did not contain any eggs or recognizable ovarioles.

In the cross between *D. tsacasi* females and *D. bakoue* males, approximately 20 F_1 females and ~15 males were obtained. Most F_1 progeny also had defective tergite cuticles, but not as severe as in the reciprocal cross. All F_1 females were back-crossed to either *D. tsacasi* or *D. bakoue* males, and laid many apparently normal eggs in both backcrosses, but no eggs hatched in either backcross. 8 F_1 females from this cross were dissected, and none were found to carry any sperm, suggesting they were defective in either mating receptivity or attractiveness. F_1 males were crossed to *D. bakoue* virgin females but produced no progeny. 5 males were dissected, and all were found to have small degenerate testes with no sperm. Thus, no F_2 progeny were obtained in either direction, despite the initial success of both crosses.

Discussion

Reproductive isolation among the African *montium* subgroup species appears to be very strong and involve both pre-mating and postzygotic isolating mechanisms. It remains possible, however, that some of these species could be hybridized under different conditions, or using other strains. In this regard, the cross between *D. tsacasi* and *D. bakoue* shows an interesting difference from an earlier report (Rafael, 1984), where no F_1 progeny were obtained. The *D. bakoue* strain used in that report was collected on the African mainland (Cameroon), whereas the strain used here was collected by Dr. Jean David on the island of Sao Tome. The latter strain could represent a divergent population of *D. bakoue*, or a cryptic species closely related to *D. bakoue* (J. David, pers. comm.). These possibilities could not be distinguished due to the lack of any other *D. bakoue* strains. It would be interesting to test other populations and strains of *D. bakoue*, *D. tsacasi*, and related species for the ability to hybridize and produce F_2 progeny. Colleagues who have any such strains are implored to send them to the author.

Acknowledgments: I would like to thank Dr. Jean David for kindly sharing the *D. bakoue* strain, and the US *Drosophila* species stock center for the other strains used in this study. I am also grateful to Dr. Emily Delaney for comments on the manuscript, and to members of the Kopp lab for letting me use their research facilities.

References: Catullo, R.A., and J.G. Oakeshott 2014, Mol. Phylogenet. Evol. 78: 275-276; Chen, C.C., M. Watada, H. Miyake, T.K. Katoh, Z. Sun, *et al.* 2013, Zoolog. Sci. 30: 1056-1062; Da Lage, J.-L., G.J. Kergoat, F. Maczkowiak, J.-F. Silvain, M.-L. Cariou, *et al.* 2007, J. Zool. Syst. Evol. Res. 45: 47-63;

Kim, B.K., T.K. Watanabe, and O. Kitagawa 1989, Jpn. J. Genet. 64: 177-190; Miyake, H., and M. Watada 2007, Genes Genet. Syst. 82: 77-88; Ohnishi, S., and T.K. Watanabe 1985, Japanese Journal of Genetics 60: 355-358; Rafael, V., 1984, Bulletin De La Societe Zoologique De France 109: 179-189; Yang, Y., Z.C. Hou, Y.H. Qian, H. Kang, and Q.T. Zeng 2012, Mol. Phylogenet. Evol. 62: 214-223; Yassin, A., E.K. Delaney, A.J. Reddiex, T.D. Seher, H. Bastide, *et al.* 2016, Curr. Biol. 26: 2412-2422; Zhang, Z., N. Inomata, M.L. Cariou, J.L. Da Lage, and T. Yamazaki 2003, J. Mol. Evol. 56: 121-130.

Electrophoretic variants of Xanthine dehydrogenase enzyme in *Drosophila malerkotliana.*

Singh, Gurvachan, and A.K. Singh. Genetics Laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi - 221005, India. Email*: aksbhu23@rediffmail.com*

Allozymes are electrophoretic variants of an enzyme. A number of enzymes are known to be polymorphic in natural populations of an organism. Xanthine dehydrogenase is an enzyme that belongs to the family of oxido-reductases involved in the oxidative metabolism of purines. This enzyme can be involved in the production of molecules called superoxide radicals. It is found to be polymorphic in a number of species of genus *Drosophila* (Singh *et al.* 1976; Kumar and Singh, 2012, 2016). Presently, we are studying the population genetics of *bipectinata* species complex that comprises four closely related species of *Drosophila* like *D. bipectinata, D. parabipectinata*, *D. malerkotliana*, and *D. pseudoananassae*. *D. malerkotliana* is one of the commonly occurring species in Indian subcontinent and it has been of ample attention to evolutionary geneticists due to its phylogenetic connection with *bipectinata* species complex (Hegde and Krishnamurthy, 1976; Jha *et al*., 1979; Tomimura *et al*., 2005; Singh and Banerjee, 2016). Genetic polymorphism in this species has been carried out by some of the researchers (Yang *et al*., 1972; Bock, 1978; Naseerulla and Hegde, 1993; Parkash *et al*., 1994; Sharma *et al*., 1993; Singh, 2015; Singh and Singh, 2015; Singh and Banerjee, 2016). Genetic polymorphism owing to allozyme variation has very sporadically been studied in this species. In the present study we are reporting the electrophoretic variants of Xanthine dehydrogenase enzyme in natural populations of *D. malerkotliana* by using native polyacrylamide gel electrophoresis.

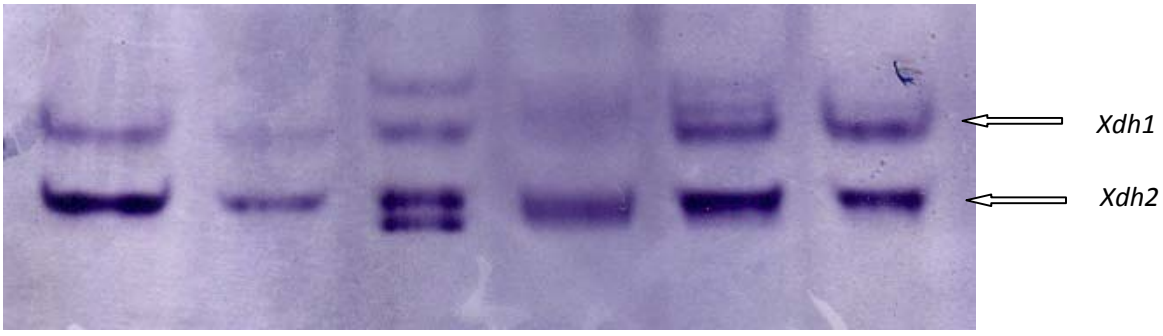

Figure 1. Xanthine dehydrogenase enzyme loci and their electrophoretic variants in *D. malerkotliana.*

For electrophoretic analysis of Xanthine dehydrogenase, single individual fly homogenate was prepared in 30 µl of 20 mM Tris buffer and the homogenate was centrifuged at 12000 rpm at 4°C for 10 minutes. Electrophoresis was performed by using polyacrylamide gel. The electrophoresis was carried out at four degree centigrade to avoid the denaturation of the enzyme. Staining of the gel was performed by using hypoxanthine as a substrate in 0.05M Tris buffer at pH 8.8 for the appearance of enzyme bands. The different