The 2LA inversion strain performed less than even the inversion free strain particularly with reference to fecundity and viability. Although inversions have an adaptive function, not all inversions are adaptive at all environments. Perhaps this is the reason for low performance of the inversion 2LA with respect to fertility and viability.

Acknowledgments: The authors are grateful to the Principal, Yuvaraja's College, Department of Zoology, University of Mysore, Mysore -570005 for providing facilities. Authors are also grateful to UGC financial support for Minor research project to Dr. S.C. Jayaramu.

References: Barker, J.S.F, 1981, [Proceeding of the 1979, Kioloa Conference] (Gibson, J.B., and J.G. Oakeshott, eds.). Australian National University: Canberra, pp. 161-184; Barker, J.S.F, and J.C. Mulley 1976, Evolution 30: 213-33; Bastock, M., 1956, Evolution 10: 421-439; Bock, I.R., and M.R. Wheeler 1972, Univ. Tex. Publ. 7213: 1-102; David, J.R., 1982, Biochem. Genet. 20: 747-761; Davis, B.J. 1964, Annals N.Y. Acad. Sci. 121: 404-427; Futch. D., 1966, Univ. Texas Publ. 6615: 79-120; Hegde, S.N., and M.S. Krishna 1997, Animal Behaviour 54: 419-426; Hegde, S.N., and N.B. Krishnamurthy 1976, Proc. Dunn. Dobzh. Symp. Genet. 36-42; Hegde, S.N., V. Vasudev, M.S. Krishna, and V. Shakunthala 1999, Entomon 24(2): 149-156; Hill, W.G., 1975, Theor. Popul. Biol. 8: 117-128; Johnson, F.M., 1971, Genetics 68: 75-95; Kessller, S., 1968, Anim. Behav. 16: 485-490; Knibb, W.B., J.G Oakeshoff, S.R. Wilson 1987, Heredity 59: 95-104; Krimbas, C.B., and J.R. Powell 1993, Edited by C.B. Krimbas and J.R. Powell. C.R.C. Press. Boca Raton FL, pp.1-52; Langley, C.H., K. Ito, and R.A Voelker 1977, Genetics 86: 447-454; Markow, T.A., 1978, Nature, London 276: 821-822; Mettler, L.E., R.A. Voelker, and T. Mukai 1977, Genetics 87: 169-176; Parsons, P.A., 1965, Experientia 27: 478-485; Rajeshwari, P., and N.B. Krishnamurthy 1969, Indian Journal of Heredity 1: 143-147; Reddy, G.S., and N.B. Krishnamurthy 1974, Dros. Inf. Serv. 51: 136-137; Singh, B.N., 1985, Nucleus 28: 169-176; Singh, B.N., and S. Chatterjee 1986, Heredity 57: 75-78; Singh, B.N., and S. Chatterjee 1987, Genetica 73: 237-242; Singh, B.N., and S.R. Singh 1999, Curr. Sci. 77: 1200-1203; Sisodia, S., and B.N. Singh 2001, Curr. Sci. 80: 1444-1447; Sisodia, S., and B.N. Singh 2005, J. Genetics 84(2): 195-216; Smith, M.J., 1956, J. Genet. 54: 261-279; Spiess, E.B., 1970, In: *Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky*. (Hecht, M.K., and W.C. Steere, eds.). Appleton-Century-Crofts. New York, pp- 315-379; Spieth, H.T, 1968, Evolutionary Biology 2: 157-191; Spieth, H.T., and J.M. Ringo 1983, In: *Genetics and Biology of* Drosophila, Vol. 3c. (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.). Academic Press, New York, pp 223-284; Zouros, E., and W. Johnson 1976, Canadian J. of Genetics and Cytology 1.

Inversion polymorphism, sexual behavior, fitness, and morphometric traits in *Drosophila ananassae*.

Jayaramu, S.C.*, and M. Prathibha. Department of Zoology, Yuvaraja's College, University of Mysore, Mysore -570005, Karnataka state, India. *Corresponding Author's E-mail: jayaramu21@gmail.com; prathibhajayaramu@gmail.com

Abstract

In the present study experimental stocks of *Drosophila ananassae* carrying 2LA, 3LA, 2LA+3LA inversions and a stock without any inversion were established from wild caught flies collected at Mysore, India. The mating activities (male courtship activities such as tapping, scissoring, vibration, circling, licking; activities of non-receptive females such as ignoring, extruding, and decamping; and mating behaviors such as courtship latency, mating latency, and copulation duration), fitness characters (fecundity and fertility), and morphometric traits (sternopleural bristles, scutellar bristles, head width, and wing length) were studied in these inversion and inversion free stocks using no choice experiment. The courtship activities and mating behaviors were quantified and compared between different strains. No relation was found between inversion stocks and male courtship activities. The carrier of two inversions (2LA+3LA) took more time to copulate but had higher fitness than inversion free stock or stock carrying single inversion. There was also no clear association between morphometric traits and inversions. The concept of inversion heterokaryotype superiority appears to be valid only to fitness traits and not for others. Key Words: *Drosophila ananassae*, courtship and mating activities, inversion, morphometric traits.

Introduction

Evolutionary response to selection depends on the amount of genetic variation present in the population of a given species. According to White (1977) chromosomal rearrangements – one of the modes of genetic variation – have played a major role in evolution, and the phenomenon has occurred many times in the evolutionary history so as to produce new variants. Inversions, in particular, found in certain species are of most frequent occurrence in *Drosophila*. A large number of investigations have been carried out on inversion polymorphism in many *Drosophila* species, and these studies have demonstrated that chromosomal polymorphism in *Drosophila* is due to paracentric inversions (Dobzhansky, 1951; Da Cunha, 1955; Krimbas and Powell, 1993). The inversions are believed to have adaptive value, and thus many workers have demonstrated superiority of inversion heterokaryotypes over homokaryotypes (Singh, and Mathew, 1993; Singh, 2008). Studies on inversion polymorphism in natural populations and role of inversions on fitness in the laboratory have confirmed the heterokaryotype superiority of inversions (Parsons, 1970; Sperlich and Pfriem, 1986; Singh and Chatterjee, 1986; Singh, 2001). Inversions also have influence on morphological traits such as sternopleural bristle numbers in natural populations (Gracia-Vazuiez *et al*., 1989; Singh and Das, 1991; Das *et al*., 1994; Yadav and Singh, 2006).

Sexual behavior and sexual selection are other important aspects of an organism's activity that have been under direct influence of natural selection. Mating propensity and success are traits that are under quantitative genetic control. Mating behavior of *Drosophila* consists of specific actions which are accompanied by orientation movements (Shorey, 1962; Limatainen, *et al*., 1992; Hegde and Krishna, 1997; Sisodia and Singh, 2005). Like propensity such actions are also genetically controlled and these actions are performed sequentially (Spiess, 1970; Shrimpton and Robertson, 1988). Since sexual behavior of males and females affects and modifies the contribution of different genotypes to the gene pool of succeeding generations, it becomes an important component of fitness. The earlier studies have clearly established the heterokaryotype superiority of inversions in terms of fitness traits such as fecundity, fertility, viability, rate of development, and so forth (Smith, 1956; Singh and Chatterjee, 1986; Singh and Mathew, 1997). These fitness traits have quantitative genetic control (Mather, 1941; Sokoloff, 1966; Sisodia and Singh, 2004). The sexual behavioral traits are also quantitative in inheritance and determine the fitness. This raises the question, whether these inversions have any effect on the sexual behavioral traits. Thus it is hypothesized that the carriers of inversion display better courtships than those of inversion-free individuals. The authors have tried to demonstrate the role of inversions on these behavioral traits and also tried to correlate with a few morphometric traits that have quantitative genetic control using an inversion-free strain and three strains of *Drosophila ananassae* carrying the inversions.

Materials and Methods

To analyze the role of inversions on mating behavior and morphometric traits, three different strains carrying 2LA, 3LA, and 2LA+3LA inversions and one inversion-free strain of *D. ananassae* were built up in the laboratory using the female flies collected from natural habitat at Mysore. These females were individually placed in vials containing wheat cream agar media (isofemale line) and, when larvae appeared, eight larvae from each vial were sacrificed to check for presence or absence of inversions in their salivary gland chromosomes. *D. ananassae* populations collected from Mysore carries two common inversions namely, 2LA and 3LA. Among these two inversions, 2LA is a sub-terminal inversion present of the left arm of second chromosome and 3LA is a terminal inversion on the left arm of the third chromosome. The wild caught individuals, therefore, would be either without inversion, or carry 2LA alone, or 3LA alone, or both 2LA+3LA. When all the eight larvae carried a given inversion, then that individual (their mother) was designated as the strain carrying that particular inversion. The adult progenies which appeared from such mothers were classified as inversions free, 2LA, 3LA, and 2LA+3LA strains. For the sake of convenience, these strains were designated as IA, IB, IC, and ID, respectively. These strains were separately maintained for six generations and at each generation, three to five larvae were used to check for the presence or absence of respective inversions. Although in each generation, the polytene chromosomes showed the presence of either inversion loop or absence of loop, because they originate from same isofemale line all progeny contained only that particular inversion homokaryotype or heterokaryotype. The adults emerged from these strains were used to build up populations for the study of variation in mating behavior and morphometric traits.

Analysis of mating behavior and fitness among four inversion phenotypes:

Virgin and bachelor flies were separated from the above cultures and allowed to age for five days. Then a bachelor male and a virgin female were introduced into an Elens Wattiaux mating chamber and allowed to copulate. The pairs which did not mate within two hours of introduction into the mating chamber were considered as unmated. The quantitative courtship acts, such as tapping, scissoring, wing vibration bouts per minute, licking, circling, ignoring, extruding, and decamping, and also mating behavior, such as courtship latency, mating latency, and copulation duration, of 30 successful pairs from each of the four strains (IA, IB, IC, and ID strains) were recorded following the procedure of Hegde and Krishna (1997).

To analyze fitness each mated pair was transferred into a vial containing wheat cream agar medium. After 24 hours, the pairs were transferred to fresh food vial, and the eggs laid in the previous vial were counted. This procedure was continued for 15 days and the total number of eggs laid and the adults emerged from each pair was recorded to determine fecundity and fertility of these strains. Mean number of eggs laid and the flies emerged per day per female was calculated and these data on the mean courtship traits, fecundity, and fertility were statistically analyzed by One way ANOVA followed by DMRT.

Analysis of morphometric characters among four inversion phenotypes:

To analyze the relation between inversions and morphometric traits, the same four strains developed as above were used. The number of sternopleural bristles, scutellar bristles, of 15 males and 15 females of IA, IB, IC, and ID strains and their head width and wing length were studied using the procedure described by Naseerulla and Hegde (1992).

Result and Discussion

Table 1 shows the mean values of courtship acts exhibited by the males and females of different inversion strains of *D. ananassae*. Tapping was observed to be higher in ID strain while lesser in IA strain. Application of one way ANOVA followed by DMRT showed that males of IA strain tapped significantly less number of times than those strains that carried inversions (Table 1). Similarly the circling activity of the males of inversion free strain was also significantly less than the carriers of inversion. It was noticed that mean wing vibration was highest in IA strain while it was least in ID. One way ANOVA followed by DMRT (Table 1) carried out on mean wing vibration showed that the mean wing vibration of IA strain was significantly greater than IC and ID strains but non-significant with IB strain. Highest scissoring was noticed in males of IC strain while lowest scissoring was found in males of IB strain. The licking activity by males was also highest in IC strain and lowest in IB. The differences of these two parameters between different strains were statistically significant by ANOVA and DMRT. The behavior of non-receptive females is also shown in Table 1. Highest ignoring was noticed in ID strain while it was lowest in IA strain. Similarly extruding by females was also highest in ID strain. Both these traits were significantly different between inversion free strain and the carriers of inversion (by One way ANOVA and DMRT). On the other hand the decamping activity performed by the females of ID female to the courting male was highest while that of the females of IB strain was lowest. These data were also statistically significant.

Table 1. Courtship acts in different inversion strains of *Drosophila ananassae* (Values are Mean ± SE.

Strain → ↓Parameters	IA (Inversion free)	IB (2LA inversion)	IC (3LA inversion)	ID (2LA+3LA Inversion)	F value
Tapping	11.00 ± 0.82^{a}	12.26 ± 1.09^{b}	11.73 ± 1.09^{a}	13.40 ± 1.26^{b}	1.80
Scissoring	13.13 ± 1.08^{b}	10.13 ± 1.30^{a}	16.27 ± 1.41^{c}	11.86 ± 1.31^{a}	8.38**
Vibration	12.46 ± 0.96^{b}	12.13 ± 1.02^{b}	9.60 ± 1.03^{a}	9.13 ± 1.18^{a}	5.23*
Circling	5.20 ± 0.30^{a}	6.53 ± 0.70^{b}	5.80 ± 0.58^{b}	5.33 ± 0.48^{a}	2.06
Licking	8.86 ± 0.76^{a}	8.46 ± 0.80^{a}	12.80 ± 1.05^{c}	10.66 ± 1.04^{b}	9.12**
Ignoring	5.00 ± 1.07^{a}	9.46 ± 1.50^{b}	6.80 ± 0.70^{a}	10.00 ± 1.53^{b}	7.26**
Extruding	1.53 ± 2.55^{a}	2.40 ± 0.49^{b}	2.13 ± 0.37^{b}	2.73 ± 0.45^{b}	3.25*
Decamping	2.66 ± 0.43^{a}	2.40 ± 0.47^{a}	3.40 ± 0.45^{a}	5.06 ± 1.11^{b}	6.42**

Note: Same superscript in each row indicates that the value is non-significant by DMRT.

*P<0.05; **P< 0.001.

In *Drosophila* many adaptive functions have been found to be associated with inversion polymorphism. Morphometric traits, fitness and certain genetic loci are associated with inversion polymorphism (Sisodia and Singh, 2001). The present study demonstrates the absence of any association of male courtship acts such as tapping, scissoring, wing vibration, licking and circling with the strains carrying inversion. Although the mean values of these courtship acts are significantly different between different inversion strains, no specific inversion or inversion-free strain showed higher activity or lower activity (Table 1). The number of times these acts performed depends on 1) the activity or vigor of males and / or 2) the receptivity of females. If the male is more active, then he performs more activity, so as to quickly mate with the female. These acts are performed to make the female sexually excited. Hence if the female is non-receptive, then also these acts are performed more. As there is no distinction between the performances of males of different strains, it seems inversions have no role to play in the performance of these acts. Thus the result indicates the absence of any association between the male courtship acts and inversions.

The behavior of non-receptive females, such as ignoring, extruding, and decamping, showed some association with inversions. Ignoring and extruding were lowest in the inversion free strain (IA) and highest in double inversion strain. Even decamping was highest in the double inversion strain. This shows that the females of inversion free strain are more receptive than the carriers of inversion, and carrying two inversion decreases the receptivity. Therefore this result contradicts the findings of Stalker (1960), Parsons (1970), Singh and Chatterjee (1986, 1987), and Singh (2008) on heterokaryotype superiority.

Table 2. Mating behavior in different inversion strains of *Drosophila ananassae* (Values are Mean ± SE).

Strain → ↓Parameters	IA (Inversion free)	IB (2LA inversion)	IC (3LA inversion)	ID (2LA+3LA Inversion)	F value
Courtship latency	50.13 ± 3.44a	46.73 ± 4.25a	41.66 ± 3.00a	69.93 ± 5.78b	8.45**
Mating latency	27.92 ± 2.72a	24.41 ± 2.99a	26.08 ± 3.19a	28.60 ± 3.30a	11.78**
Copulation duration	3.76 ± 0.14^{a}	4.08 ± 0.18^{b}	3.60 ± 0.13^{a}	4.11 ± 0.10^{b}	2.99*
Fecundity No./day/female	45.53 ± 4.81a	57.53 ± 5.33a	122.06 ± 5.35b	131.53 ± 11.95b	49.10**
Fertility No/day/female	31.53 ± 4.15^{a}	24.26 ± 2.76^{a}	69.80 ± 9.93b	83.86 ± 11.88b	12.68**

Note: 1) Same superscript in each row indicates that the value is non-significant by DMRT. 2) Courtship latency is measured in seconds while mating latency and copulation duration are measured in minutes. 3) *P<0.05; **P< 0.001.

Variation in mating behavior and fitness of inversion strains:

Table 2 shows mean courtship latency, mating latency, copulation duration, fecundity, and fertility performed by the flies of different strains of *D. ananassae*. It is noticed that courtship latency, mating latency, and copulation duration were highest in ID, which is a strain carrying two inversions. The strains carrying only one inversion, *i.e.*, either IB or IC, showed the lowest values. One way ANOVA followed by DMRT applied on mean courtship latency of different strains (Table 2) showed that these behavioral traits varied significantly between them. This means the females of strains carrying single inversion are sexually more active and receptive than either inversion free or double inversion strains. The table also shows that fecundity was lowest in IA strain, increased in the single inversion strains of IB and IC and highest in ID. Similar was the observation with reference to fertility except that IB strain has the lowest fertility. Both these data on mean fecundity and fertility subjected to one way ANOVA followed by DMRT showed significant variation.

Courtship latency and mating latency are the indicators of mating speed and copulation duration is the time available for males to transfer the sperms to the female genital tract (Spiess and Langer, 1964; Sisodia and Singh, 1996). It is noticed that courtship latency, mating latency, and copulation duration were highest in ID, which is a strain carrying two inversions. The double inversion strain does not exhibit any superiority over inversion free strain or the strains carrying single inversions. On the other hand strains carrying only one inversion, *i.e.*, either IB or IC showed the lowest values (Table 2). This means the females of strains carrying single inversion are sexually more active than either inversion free or double inversion strains. The observation of heterozygote superiority is applicable provided the fly has only single inversion. However this observation contrasts that Stalker (1960) who has demonstrated concentration of inversions on the same chromosome.

Table 2 also shows that fecundity was lowest in IA strain, increased in the single inversion strains of IB and IC and highest in ID. Similar was the observation with reference to fertility except that IB strain has the lowest fertility. Both these data on mean fecundity and fertility subjected to one way ANOVA followed by DMRT showed significant variation. It is evident that although the carriers of inversions have no heterozygote superiority, with reference to courtship acts, mating speed, they exhibit this phenomenon with reference to fecundity and fertility. This observation supports the work of earlier authors (Singh and Mathew, 1997; Krishna and Hegde, 2003; Sisodia and Singh, 2001, 2005).

Variation in morphometric traits of inversion strains:

The data on morphometric characters such as sternopleural bristles, scutellar bristles, head width, and wing length in IA, IB, IC, and ID strains are provided in Table 3. Mean number of sternopleural bristles of IB strain was highest while, it was lowest in IC. Mean number of scutellar bristles, head width, and wing length was highest in ID strain. However, lowest number of scutellar bristles was noticed in the inversion-free strain. Further the head width and wing length were lowest in the carriers of 2LA inversion. The data on these morphometric traits when subjected to one way ANOVA showed that they are significantly different between different strains.

Table 3. Morphometric traits in different inversion strains of *Drosophila ananassae* (Values are Mean ± SE).

Strain → ↓Parameters	IA (Inversion free)	IB (2LA inversion)	IC (3LA inversion)	ID (2LA+3LA Inversion)	F value
Sternopleural bristles	6.73 ± 0.16^a	7.03 ± 0.11^b	6.46 ± 0.09^a	6.90 ± 0.14^b	11.31**
Scutellar bristles	4.30 ± 0.08^a	4.40 ± 0.09^a	4.50 ± 0.09^a	4.60 ± 0.09^b	6.86**
Head width	0.82 ± 0.01^b	0.76 ± 0.01^a	0.77 ± 0.01^a	0.83 ± 0.01^b	6.96**
Wing length	1.97 ± 0.01^a	1.95 ± 0.02^a	2.01 ± 0.02^b	2.03 ± 0.01^b	3.06*

Same superscript in each row indicates that the value is non-significant by DMRT.
*P<0.05; **P< 0.001.

Heterokaryotypic superiority of inversions is also evident in the morphometric characters such as sternopleural bristles, scutellar bristles, head width, and wing length (Hegde and Krishna, 1997; Sokoloff, 1966; Shrimpton and Robertson, 1988). Mean number of scutellar bristles, head width, and wing length was highest in ID strain, which has two inversions (Table 3). The strain carrying 3LA (IC strain) also performs well compared to inversion-free strain. Only in 2LA inversion strain mean head width and wing length were less than others. Thus the present study demonstrates the heterokaryotypes superiority of only certain fitness traits and not all. Further no such superiority or association between the presence of inversions and the courtship behavior is noticed in *D. ananassae*.

Acknowledgment: The authors are grateful to Principal, Yuvaraja's College, University of Mysore, for providing facilities, and grateful to University Grants Commission for awarding minor research project.

Reference: Da Cunha, A.B., 1955, Advances in Genetics 7: 93-138; Das, A., S. Mohanty, and B.B. Parida 1994, Heredity 73: 405-409; Dobzhansky, Th., 1951, 3rd ed., Columbia University Press, New York; Garcia-Vazquiez, E., F. Sanchez-Refusta, and J. Rubio 1989, Heredity 67: 183-187; Hegde, S.N., and M.S. Krishna 1997, Animal Behavior 54: 419-426; Krimbas, C.B., and J.R. Powell 1993, (Krimbas, C.B., and J.R. Powell, eds.). C.R.C. Press, Boca Raton, FL. pp.1-52; Krishna, M.S., and S.N. Hegde 2003, Italian Journal of Zoology 70: 47-52; Limatainen, J., A. Hoikkala, J. Aspi, and P.H. Welbergen 1992, Anim. Behav. 43: 35-48; Mather, K., 1941, Genetics 4: 159-193; Naseerulla, M.K., and S.N. Hegde 1992, Bulletino de Zoologica 59: 367-370; Parsons, P.A., 1970, Theor. Appl. Genet. 40: 337-340; Shrimpton, A.E., and A. Robertson 1988, Genetics 118: 437-443; Shorey, P.M., 1962, Science 137: 677-678; Singh, B.N., 2001, Ind. J. Exp. Biol. 39: 611-622; Singh, B.N., 2008, Curr. Sci. 94 (4): 459-464; Singh, B.N., and S. Chatterjee 1986, Heredity 57: 75-78; Singh, B.N., and S. Chatterjee 1987, Genetica 73: 237-242; Singh, B.N., and A.

Das 1991, Evol. Biol. 5: 185-200; Singh, B.N., and S. Mathew 1993, Evol. Biol. 7: 313-325; Singh, B.N., and S. Mathew 1997, Curr. Sci. 72: 112-114; Sisodia, S., and B.N. Singh 1996, Zool. Stud. 35: 25-29; Sisodia, S., and B.N. Singh 2001, Curr. Sci. 80: 1444-1447; Sisodia, S., and B.N. Singh 2004, Heredity 60: 269-272; Sisodia, S., and B.N. Singh 2005, J. Genetics 84 (2): 195-216; Smith, M.J., 1956, J. Genet. 54: 261-279; Sokoloff, A., 1966, Evolution 20: 49-71; Sperlich, D., and P. Pfriem 1986, In: *The Genetics and Biology of* Drosophila, Vol-3e. (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.). Academic Press, New York, pp. 257-309; Spiess, E.B., 1970, In: *Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky*. (Hecht, M.K., and W.C. Steere, eds.). Appleton-Century-Crofts, New York, pp. 315-379; Spiess, E.B., and B. Langer 1964, Evolution 18: 430-444; Stalker, H.D., 1960, Genetics 45: 95-114; White, M.J.D., 1977, Cambridge University Press, 1973. Reprinted by Vikas. Publ. House. PVT, LTD, 3rd Edition, pp. 231-284; Yadav, J.P., and B.N. Singh 2006, J. Zool. Syst. Evol. Res. 44: 323-329.

The first record of *Zygothrica orbitalis* (Sturtevant, 1916) for the state of Rio Grande do Sul and the southernmost limits for seven species of Drosophilidae (Insecta: Diptera).

Valer, Felipe B., Alexandre Schneid Neutzling, Flávio Roberto Mello Garcia, Marco Silva Gottschalk, and Monica Laner Blauth. Departamento de Ecologia, Zoologia e Genética, Instituto de Biologia, Universidade Federal de Pelotas. CP 354, CEP 96010-900. Pelotas, RS, Brazil. Phone: 55 53 3275 7340. Fax 55 53 3275 7335. E-mail: fbvaler@gmail.com

Introduction

Although the state of Rio Grande do Sul (RS) has been one of the most well-surveyed Brazilian states for Drosophilidae, with 86 species records, most of these surveys were conducted in the Atlantic Forest Biome, whereas the Pampa Biome has been less explored in terms of species occurrence (Gottschalk *et al*., 2008; Poppe *et al.*, 2012). Furthermore, most collections were made exclusively using banana-bait traps in the city of Porto Alegre (Silva *et al*., 2005; Garcia *et al*., 2008; Garcia *et al*., 2012) and its surroundings, *e.g*., at Itapuã State Park (Valente and Araújo, 1991) and in fields of native grasses at Guaíba (Saavedra *et al*., 1995). Recently, collections were made using the same bait in a transitional area between the Pampa and Atlantic Forest Biome by Hochmüller *et al.* (2010) and near the northern border of the Pampa Biome by Poppe *et al*. (2012). In this study, we aim to record the Drosophilidae species trapped in McPhail traps with Karo® Syrup bait in the Pampa Biome, southern Brazil. These records show the southern-most limit of distribution for eight species and one new record for RS.

Materials and Methods

In January 2010, flies were collected in a Restinga forest area of 23 ha belonging to the Federal Preservation Unit Horto Botânico Irmão Teodoro Luís (HBITL) (31°48'54"S; 52°25'48"W), southern RS, Brazil (Figure 1).

HBITL is located in the Pampa Biome and has a strong influence of Seasonal Forest Semidecidual Submontane. The climate is Mesothermal Bland Superhumid, without distinct dry