Distribution and Hybridization between *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) in Argentina

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To better understand the distribution of Culex pipiens *and* Cx. quinquefasciatus *in Argentina, samples were collected from six localities situated in a North-South line from Castelli (Chaco Province) to Puerto Madryn (Chubut Province). Identification was based on the morphology of male genitalia. Only* Cx. quinquefasciatus *was found in Castelli and Esperanza, while in Rosario, 95.3% belonged to this species and 4.7% represented hybrid forms. Southern samples included only* Cx. pipiens. *With the purpose of verifying if* Cx. pipiens *and* Cx. quinquefasciatus *hybridize, different crosses between the two species were performed. All crosses produced viable egg rafts. Hatching ranged from 70 to 100%, except in one cross, female* Cx. pipiens *x male* Cx. quinquefasciatus, *where a high incompatibility was observed (11.1% hatch). The F_1 hybrids obtained from all crosses were fertile. The finding of hybrid forms in nature can be interpreted as evidence for subspecific status of* Cx. pipiens *and* Cx. quinquefasciatus *in Argentina.*

Key words: *Culex pipiens* - *Culex quinquefasciatus* - geographical distribution - intergradation area - crossing experiments

The *Culex pipiens* group includes closely related mosquitoes with a wide geographical distribution. The medical and veterinary importance of these insects lies in the fact that some species are vectors of pathogens that cause different diseases (Laven 1967), and some are important domestic pests. Different subgroups have been recognized (Belkin 1962, Sirivanakarn 1976, Harbach 1988). Within the *pipiens* subgroup, the species *Cx. pipiens* L. has an Holarctic distribution and inhabits areas of temperate climate in the southern hemisphere, including Australia, Africa and South America. *Cx. quinquefasciatus* Say occurs throughout the tropics and subtropics of the world (Mattingly 1951, Laven 1967).

Even though *Cx. pipiens* and *Cx. quinquefasciatus* populations are geographically separated, when their ranges overlap, hybridization occurs with the resulting appearance of intermediate forms. Areas of hybridization occur in North America (Sundararaman 1949, Barr 1957, Cheng 1976, Jakob et al. 1980), northern Japan (Bekku 1956, Sasa et al. 1963, Ishii 1980), the Middle East (Edwards 1921, Harbach 1985, 1988), and southeastern Australia (Dobrotworsky 1967, Barr 1982). These authors (except Harbach 1985, 1988) consider that these taxa belong to a single polytypic species, *Cx. pipiens*, with *quinquefasciatus* and *pipiens* as subspecies.

In South Africa, Jupp (1978) and Donaldson (1979) have pointed out that these taxa coexist in nature without evidence of hybridization. Moreover these forms do not interbreed under laboratory conditions, so they proposed that *Cx. pipiens* and *Cx. quinquefasciatus* are separate species. Sirivanakarn and White (1978), Harbach et al. (1985) and Harbach (1988) also consider these forms as separate species.

Information about the distribution of these taxa in Argentina indicates that *Cx. quinquefasciatus* occurs from the provinces of Buenos Aires and Mendoza northwards, whereas *Cx. pipiens* is found from Buenos Aires southwards to the Santa Cruz Province (Duret 1953, Mitchell et al. 1984). However, the possible intergradation and extension of overlapping areas between the two forms in this country are not known. Brewer et al. (1987) reported the presence of intermediate forms in Córdoba Province.

The aim of this study was to determine the geographical distribution of *Cx. pipiens* and *Cx.*

This work was supported partially by grants from the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR), Argentina. CNG is a Career Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina.
Received 18 August 1994
Accepted 16 February 1995

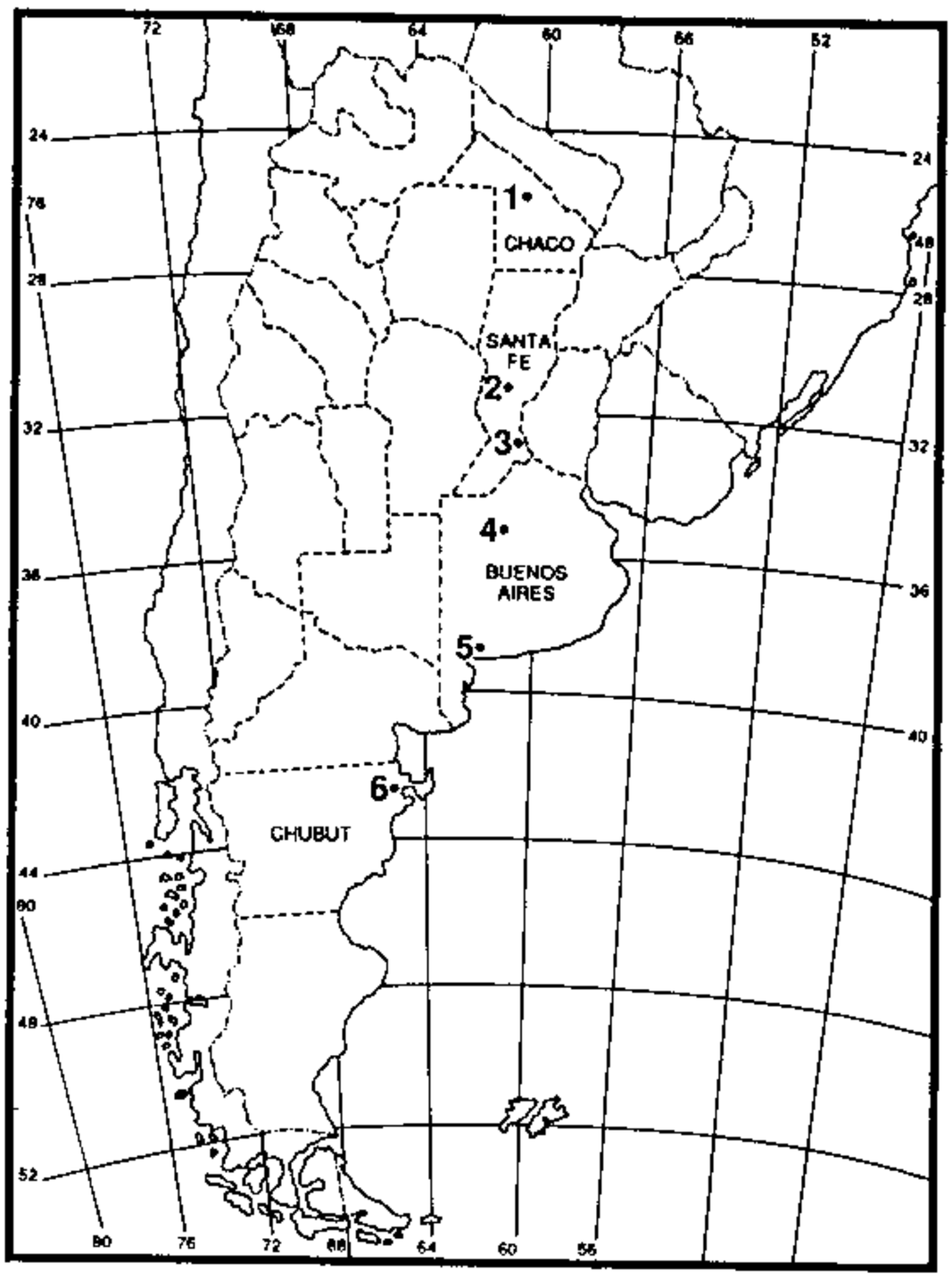
quinquefasciatus in Argentina along a north-southern line in order to detect a possible hybridization zone, and to determine if these forms hybridize in the laboratory.

MATERIALS AND METHODS

Geographical distribution - Samples were taken from Castelli, 25° 53'S (Chaco Province); Esperanza, 31° 26'S, and Rosario, 32° 56'S (Santa Fe Province); 9 de Julio, 35° 18'S and Bahía Blanca, 38° 37'S (Buenos Aires Province); and Puerto Madryn, 42° 46'S (Chubut Province) during the summer of 1989-1992 (Fig.). Additional samples were obtained in April (autumn) and October (spring) 1989 in Esperanza, and in June (winter) 1989 at Castelli. Blood fed females were collected by means of chicken-baited can traps and then taken to the laboratory. In order to obtain egg rafts, females were placed individually in plastic tubes with wet cotton and filter paper at the bottom. Tubes were checked daily and egg rafts obtained were placed in plastic trays and reared as individual progenies (25±3°C; 14:10 L/D photoperiod). Larvae were fed with commercial rodent food until adults emerged. *Cx. pipiens* and *Cx. quinquefasciatus* were identified by the morphology and morphometry (DV/D ratios) of the male genitalia of five adults from each progeny brood. DV/D ratios are the only reliable means of distinguishing these species. Males with a DV/D ratio of 0.20 or less were identified as *Cx. pipiens* and individuals with values of 0.40 or higher were identified as *Cx. quinquefasciatus*. Those with values between 0.20 and 0.40 were classed as intermediates (Sundararaman 1949).

Crossing experiments - *Culex pipiens* and *Cx. quinquefasciatus* from Rosario and Bahía Blanca were used for the crossing experiments. Individuals from Córdoba Province were also used. Adult progeny were obtained as previously described and separated daily according to sex and species.

Males and females were isolated before sexual maturation to prevent interbreeding among progeny of single mothers. Virgin males and females were placed in cages (30x30x30cm) according to the following crosses: females *Cx. pipiens* (Bahía Blanca or Córdoba) x males *Cx. quinquefasciatus* (Córdoba or Rosario) and their reciprocals (Table I). Adults were fed on a sugar solution (10%) during seven days, then a blood meal (chicken) was offered prior to starvation for 24 hr. This meal was offered twice a week. In order to obtain egg rafts, plastic trays with water were placed inside the cages. Rafts were collected daily and reared as previously described. Hybrids obtained were also reared to test their fertility by interhybrid crosses.



Location of *Culex pipiens* and *Cx. quinquefasciatus* collection sites: 1. Castelli; 2. Esperanza; 3. Rosario; 4. 9 de Julio; 5. Bahía Blanca; 6. Puerto Madryn.

RESULTS AND DISCUSSION

Geographical distribution - Samples belonging to the northern localities (Castelli and Esperanza) included only *Cx. quinquefasciatus*. In Rosario, although 95.3% of species were *Cx. quinquefasciatus*, a low percentage (4.7%) of hybrids was observed. In 9 de Julio, 96.8% of the individuals were identified as *Cx. pipiens*, but one *Cx. quinquefasciatus* was detected. All individuals captured in southern areas (Bahía Blanca and Puerto Madryn) were *Cx. pipiens* (Table II).

Our findings agree with the observations made by Brewer et al. (1987) which indicate the presence of hybrids in Argentina. These authors mention Córdoba Province as a possible intergradation area. Our results extend this area to the south of Santa Fe Province.

Even though intermediate forms were not detected in 9 de Julio, one specimen of *Cx. quinquefasciatus* was obtained. Its presence could be possibly due to passive transport by man. More extensive sampling is needed in order to determine the possible existence of hybrids in this area.

Crossing experiments - Viable eggs were obtained from all crosses. Crosses involving female *Cx. pipiens* from Córdoba and male *Cx. quinquefasciatus* from Rosario (cross no. 2) showed hatching rates of 91.1%, and 88% in the reciprocal cross

TABLE I

Results of crosses between *Culex pipiens* (*Cx.p.*) and *Cx. quinquefasciatus* (*Cx.q.*) from different populations of Argentina

	Crosses			N		F_1		F_1 x F_1	
				Female	Male	Egg rafts		Egg rafts	
						Obt.	Hatched	Obt.	Hatched
						N	%	N	%
	Female *Cx. p.* x Male *Cx. q.*								
1	BB	x	ROS	331	213	81	11.1	10	100
2	CBA	x	ROS	61	82	12	91.7	-	-
3	BB	x	CBA	29	25	18	100	23	60.9
	Female *Cx. q.* x Male *Cx. p.*								
1'	ROS	x	BB	180	319	61	70.5	50	74
2'	ROS	x	CBA	61	60	25	88	16	56.2
3'	CBA	x	BB	45	47	14	92.8	8	100

BB: Bahía Blanca (Buenos Aires); ROS: Rosario (Santa Fe);
CBA: Córdoba (Córdoba)
Obt.: obtained egg rafts; -: not scored

TABLE II

Presence of *Culex pipiens* (*Cx.p.*), *Cx. quinquefasciatus* (*Cx.q.*) and hybrids in six localities of Argentina

Locality	Egg rafts (N)	*Cx. p.* %	Hybrids %	*Cx. q.* %
Castelli (Chaco)[a]	29	0	0	100
Esperanza (Santa Fe)	26	0	0	100
Rosario (Santa Fe)	86	0	4.7	95.3
9 de Julio (Buenos Aires)	32	96.8	0	3.3
Bahía Blanca (Buenos Aires)	77	100	0	0
Puerto Madryn (Chubut)	15	100	0	0

[a]: names in parenthesis are provinces

(cross no. 2'). High percentages of eclosion were also obtained in crosses 3 and 3'. The offspring of all crosses proved to be fertile, hatching being between 56 and 100% (Table I). An important difference in the hatch rate was observed in crosses between individuals from Bahía Blanca and Rosario, being 11.1% in the cross female *Cx. pipiens* from Bahía Blanca x male *Cx. quinquefasciatus* from Rosario, and 70.5% in the reciprocal cross.

Unidirectional incompatibility is common in *Cx. pipiens* (French 1978). Yen and Barr (1973)

showed that this phenomenon is the manifestation of an asymmetrical interaction between parental cytoplasms containing a rickettsia-like symbiont called *Wolbachia pipientis*. Each population of *Cx. pipiens* has developed its own clone of microorganisms. This coadapted combination of mosquito and *Wolbachia* is interpreted as producing a spectrum of mating types. Nevertheless, DNA sequence data from *Wolbachia* resident in different genera, species and strains of mosquitoes (O'Neill et al. 1992, Rousset et al. 1992) indicate low levels of variation among them, leading these authors to conclude that all insects tested harbor a monophyletic assemblage of the endosymbionts. Kambhampati et al. (1993), studying different populations of *Aedes albopictus*, found that individuals did not harbor different strains of *Wolbachia*. Nigro (1991) and Kambhampati and Rai (1991) ruled out any correlation between incompatibility and mitochondrial genome. We plan to conduct further studies to elucidate an explanation for the partial incompatibility observed in one of the crosses in this study. The finding of hybrids in the field and their production in the laboratory can be interpreted as evidence for the subspecific status of *Cx. pipiens* and *Cx. quinquefasciatus* in Argentina.

ACKNOWLEDGMENTS

To Dr Marta Sabattini for her assistance, to Dr Antonio Blanco for his critical revision of the manuscript, and to Dr Gabriela Avilés and José Daffner who collected three of the samples.

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