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Cross-breeding studies in seven *Artemia franciscana* strains from Mexico

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Cross-breeding experiments were performed on seven Mexican strains of *Artemia franciscana* to determine their status of reproductive isolation. The populations used in this study were: Yavaros (YAV), Ohuira (OHUI), Juchitan (JUCH), Real de las Salinas (R.SAL), Cuatro Cienegas (C.CIEN), Las Salinas de Hidalgo, San Luis Potosi (SLP) and Texcoco (TEX). Each population was cultured in a 40-l tank. Feeding consisted of a mixture of *Tetraselmis* sp. and *Dunaliella* sp. When the organisms had reached the pre-adult stage, males and females were separated. For cross-breeding experiments, one female from one population and two males from another population were placed in a 250-ml flask; the same procedure was followed for the reciprocal crosses. Once the presence of nauplii (F_1) was observed, they were placed in another flask until they were sexually mature. From the 42 cross-breeding tests performed, only seven presented 100% reproductive success, while 15 were not successful. For F_2, the number of successful cross-breedings dropped drastically, showing increased sterility. Only the cross-breeding involving female TEX/males R.SAL maintained high nauplii production in F_1 and F_2 (445 and 401, respectively). We can conclude that the studied Mexican populations of *Artemia franciscana* are in the process of building post-zygotic reproductive isolation (hybrid breakdown), caused by the ecological preferences and/or adaptation of each population to its specific habitat. This might be reflected in their genotype, inducing pre- and post-mating alterations.

Key Words: *Artemia franciscana*, Mexico, cross-breeding, reproduction, strains.

INTRODUCTION

Species characterization in the genus *Artemia* involves different methodologies. The most frequently used are: morphometry, allozyme analysis, and reproductive studies based on cross-fertility tests (Hontoria & Amat, 1992).

Cross-breeding studies have been very useful to detect the degree of reproductive isolation in specific groups (Dobzhansky, 1951; Mayr, 1969; Correa *et al*., 1993).

Gajardo *et al.* (2002) indicated the existence of seven bisexual species: *Artemia salina, A. urmiana, A. sinica, Artemia* sp. (Kazakhstan), *A. tibetiana, A. persimilis,* and *A. franciscana.* These authors mentioned that the name of *A. tunisiana* was used instead of *A. salina,* which is now recognized as the only bisexual species located in the Mediterranean area.

The first work on cross-breeding with different *Artemia* populations was performed by Bowen (1965). It was not until 1980 that Bowen *et al*. performed a work of related populations with similar characteristics, and considered them as sibling species. In the same year, Browne (1980a,b) determined the pattern of reproduction in *Artemia* and achieved cross-breeding of bisexual and parthenogenetic populations.

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Gallardo & Castro (1987) performed the first study of cross-breeding with Mexican *Artemia* populations. These authors observed that although the studied populations gave fertile descendants, the number of successful cross-breedings was low.

Correa *et al*. (1993) performed a hybridization study with two *Artemia* populations, one from Baja California (Mexico) and the other from the San Francisco Bay (SFB). They observed fertile descendants from the cross-breeding, although success in the experiments varied from 7% to 80%. Correa & Tapia (1998) studied the reproductive behavior of the population of *A. franciscana* from San Quintín (Baja California) through cross-breeding with the population of Yavaros (Sonora) resulting in 73% success when the male was from San Quintín, but it decreased to 55% when the female was from San Quintín. The authors suggested a principle of specific differentiation.

Castro *et al*. (1999) studied the variations of the reproductive patterns of the *Artemia* populations from Yavaros (Sonora), Ceuta Bay (Sinaloa) and SFB. They found difficulties in reproduction during cross-breeding among the Mexican populations while cross-breeding between Mexican *Artemia* and *Artemia* from SFB increased production significantly. Castro *et al*. (1989) performed cross-breeding studies with populations from Yavaros, Texcoco, San Crisanto (Yucatan), Ceuta Bay, and San Luis Potosi and observed that the most successful cross-breedings were achieved between the San Luis Potosi and the San Crisanto populations. The other populations showed decreased cross-breeding success.

Gajardo *et al*. (1998) performed cross-breeding studies with *Artemia* populations from South America (Chile, Argentina, Peru, and Brazil) and the SFB population and reported a cross-breeding success of up to 90%. Gajardo *et al*. (2002) pointed out the evolution and speciation aspects undergone by the genus *Artemia* along its existence and discused the different identified species of *Artemia.*

Zúñiga *et al*. (1999) performed cross-breeding studies with Chilean populations and found problems in reproduction, as they obtained F_2 descendants.

Pilla & Beardmore (1994) demonstrated that *A. urmiana*, *A. sinica,* and *Artemia* from Kazakstan populations presented a great morphological and genetic differentiation. However, in laboratory conditions, these populations were interfertile without showing reduced viability in the formed hybrids. These authors also pointed out the complete infertility when crossing the populations from Europe with *Artemia franciscana* from America. Truong-Trong (1995) demonstrated the absence of genetic barriers among *A. urmiana*, *A. sinica,* and *A.* from Kazakstan, observing that their cross-breeding could reach F_6 generation. These authors also pointed out that no genetic barriers have been observed that cause interruption of the gene flow among the populations of Eastern Europe. Abatzopoulos *et al.* (1998) pointed out the presence of interfertility between *A. salina* and *A. franciscana.* Their results revealed one exception; they got, in F_1, 13 sterile nauplii when mating female *A. tibetiana* and male *A. franciscana.*

All these studies reveal that it is necessary to consider a combination of methods and disciplines in order to characterize new populations of *Artemia*, by morphology, genetic distances, cytogenetics, ecological isolation, and through cross-breeding or hybridization.

The reproductive capacity and reproductive isolation are the two most interesting issues in evolutionary ecology. Reproductive isolation is a key factor in the biological species concept (Mayr, 1969). However, the speciation process is considered equivalent to the development of barriers that cause separation of populations. This is why hybridization between populations can have low or high evolutionary significance, since the formed hybrids may present low or high strength to resist the environment from which their progenitors have preceded (Gajardo *et al.,* 2001).

The objective of the present work was to characterize *Artemia franciscana* from seven Mexican populations, taking into account the problem of the Mexican populations to interbreed successfully. Besides, the state of post-zygotic reproductive isolation is discussed.

MATERIAL AND METHODS

Artemia *populations*

The sites of *Artemia franciscana* populations studied in this work are presented in Table 1 and Fig. 1.

From each of the studied populations, 2.0 g cysts were hatched in artificial seawater of 40 gl^{-1} salinity, temperature of $24 \pm 2\,^{\circ}C$, and constant light during the first 24 hours. The obtained nauplii were placed for their culturing in 160-l plastic cylinders with water at a salinity of 60 gl^{-1}, temperature of $24 \pm 2\,^{\circ}C$, and a density of 50 $org \cdot l^{-1}$. Feeding consisted of a

TABLE 1. Geographical locations of *Artemia* sites in Mexico

Name	Abbreviations	Geographic coordinates
Yavaros, Sonora	YAV	26°40′N; 109°35′W
Ohuira, Sinaloa	OHUI	25°36′N; 109°02′W
Juchitán, Oaxaca	JUCH	16° - 16°39′N; 94°45′ - 95°06′W
Real de las Salinas, Campeche	R. SAL	20°02′N; 90°14′W
Cuatro Ciénegas, Coahuila	C.CIEN	29°36′N; 99°20′W
Las Salinas de Hidalgo, San Luis Potosí	SLP	22°39′N; 101°43′W
Texcoco, Edo. de México	TEX	19°32′N; 99°00′W

Castro *et al.* (2000)

FIG. 1. Geographical locations of *Artemia* sites in Mexico.

mixture of two microalgae (*Tetraselmis* sp. and *Dunaliella* sp.), at a ratio of 1:1. These conditions were maintained until the animals reached the pre-adult stage, at which morphological differentiation began. The males were placed in a 4-l flask, with 3 l of water at 60 gl^{-1} of salinity and the same culture conditions. For the females, 10 of them were placed in 1-l flasks, with 800 ml of water at 60 gl^{-1} salinity and the same cultivation conditions.

For cross-breeding, one female from a population and two males from another population were placed in a 250 ml flask, while the same procedure was followed for the reciprocal crosses (Table 2).

Each cross-breeding was observed daily. As soon as the nauplii appeared (F_1), they were placed in 4-l flasks, with water at 60 gl^{-1} salinity; 24 ± 2°C temperature, constant light, and microalgae until they reached adulthood.

RESULTS

Tables 3 and 4 depict the reproductive success and the numbers of produced nauplii (F_1) per cross-breeding among the studied populations; 15 cross-breedings were not successful, not even mating was observed between the formed couples.

TABLE 2. Experimental crosses between Mexican populations

Populations		Populations	
Female	Male	Female	Male
Yavaros	Ohuira	Cuatro Cienegas	Yavaros
	Juchitan		Ohuira
	Real de las Salinas		Juchitan
	Cuatro Cienegas		Real de las Salinas
	San Luis Potosi		San Luis Potosi
	Texcoco		Texcoco
Ohuira	Yavaros	San Luis Potosi	Yavaros
	Juchitan		Ohuira
	Real de las Salinas		Juchitan
	Cuatro Cienegas		Real de las Salinas
	San Luis Potosi		Cuatro Cienegas
	Texcoco		Texcoco
Juchitan	Yavaros	Texcoco	Yavaros
	Ohuira		Ohuira
	Real de las Salinas		Juchitan
	Cuatro Cienegas		Real de las Salinas
	San Luis Potosi		Cuatro Cienegas
	Texcoco		San Luis Potosi
Real de las Salinas	Yavaros		
	Ohuira		
	Juchitan		
	Cuatro Cienegas		
	San Luis Potosi		
	Texcoco		

TABLE 3. Reproductive success (%) for each parental mating

Females	Males						
	YAV	OHUI	JUCH	R.SAL	C.CIEN	SLP	TEX
YAV		80	40	20	100	20	40
OHUI	20		0	0	60	0	100
JUCH	40	0		0	100	0	40
R.SAL	20	0	100		60	0	40
C.CIEN	40	0	80	40		0	100
SLP	0	0	0	0	0		60
TEX	40	20	100	100	60	60	

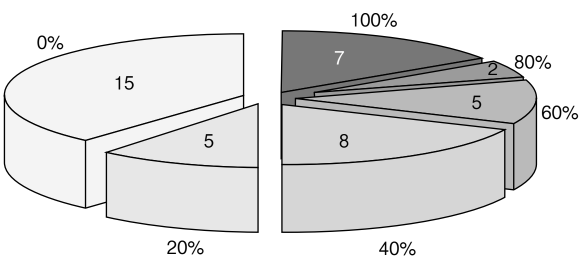

FIG. 2. Reproductive success (%) in parental crosses between Mexican populations.

TABLE 4. Naupliar production during parental crosses

Females	Males						
	YAV	OHUI	JUCH	R.SAL	C.CIEN	SLP	TEX
YAV		84	38	27	93	2	7
OHUI	48		No mating	No mating	101	No mating	120
JUCH	60	No mating		No mating	75	No mating	106
R.SAL	7	No mating	133		71	No mating	53
C.CIEN	62	No mating	102	93		No mating	170
SLP	No mating	No mating	No mating	No mating	No mating		78
TEX	12	20	219	445	27	57	

TABLE 5. Reproductive success (%) for each F_1 mating

Females	Males						
	YAV	OHUI	JUCH	R.SAL	C.CIEN	SLP	TEX
YAV		80	0	20	100	20	0
OHUI	20		0	0	60	0	100
JUCH	0	0		0	100	0	0
R.SAL	0	0	0		0	0	0
C.CIEN	0	0	0	0		0	0
SLP	0	0	0	0	0		60
TEX	0	0	100	100	60	60	

TABLE 6. Naupliar production during F_1 crosses

Females	Males						
	YAV	OHUI	JUCH	R.SAL	C.CIEN	SLP	TEX
YAV		148	0	7	92	0	0
OHUI	54		No mating	No mating	141	No mating	140
JUCH	0	No mating		No mating	85	No mating	0
R.SAL	0	No mating	0		0	No mating	84
C.CIEN	0	No mating	0	0		No mating	0
SLP	No mating	No mating	No mating	No mating	No mating		115
TEX	0	0	154	401	60	71	

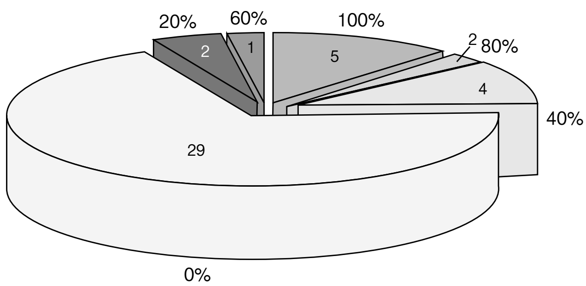

FIG. 3. Reproductive success (%) in F1 crosses between Mexican populations.

Table 3 shows the cross-breedings that had 100% success, these were: YAV ♀/♂ C.CIEN; OHUI ♀/♂ TEX; JUCH ♀/♂ C. CIEN; R. SAL ♀/♂ JUCH; C.CIEN ♀/♂ TEX; TEX ♀/♂ JUCH and with ♂ R. SAL.

From the 42 cross-breedings among the populations, only seven presented 100% reproductive success; two, 80%; five, 60%; eight, 40%; five, 20%; and 15 were not successful (Fig. 2).

The cross-breeding that produced the most nauplii (445) was TEX♀ and R. SAL♂. From the other cross-breedings, six produced 101 to 170 nauplii, and the remainder cross-breedings produced less than 100 nauplii. The lowest production was attained by ♀YAV x ♂SLP with only two nauplii (Table 4).

It was found that those hybrids that reached sexual maturity and cross-bred again at F_2 depicted a decreased number of successful cross-breedings, since those descendants of the 100% success cross-breeding went down to five; only one depicted 80%; one, 60%; two, 20%; and 29, 0% (Table 5, Fig. 3).

Table 6 presents the production of nauplii. This production was high for the ♀TEX/♂R.SAL cross-breeding, but the amount diminished markedly for the other cross-breedings. It is worthwhile pointing out, that in some F_2 cross-breedings, despite mating, no nauplii production was obtained.

DISCUSSION

Cross-breeding difficulties exist among the studied Mexican populations, mainly with the SLP population, which yielded only very low reproduction success when cross-bred with the TEX population and low number of descendants. These findings indicate that this population already presents reproductive isolation with most of the populations.

Other populations that present few cross-breedings are OHUI and JUCH, when the female is from OHUI and the male is from YAV. Although OHUI and YAV are close geographically, their reproduction success was only 20%, but it increased considerably (80%) when the female was from YAV. This also happened when using C.CIEN and TEX males with 100% success, but decreased to 20% when using C.CIEN males and TEX females (60%). The JUCH population also showed mating with three populations, YAV, C.CIEN and TEX. Note that JUCH female/C.CIEN male cross-breeding reached 100% of reproduction success. The same occurred between TEX females and JUCH males.

The two populations that cross-bred successfully with all the others were YAV and TEX. The first one gave 100% of reproduction success, only when crossing YAV females with C.CIEN males. Mating success decreased consideraly (40%) when the female belonged to C.CIEN and the male to YAV. The second population, TEX, showed the highest level of reproductive success when crossing TEX females with JUCH and R.SAL males. However, with females of the latter two populations, only 40% of success was reached. Likewise, 100% success was obtained with OHUI and C.CIEN females and TEX males. It is noteworthy that the TEX population was the only one crossing with either females or males of the SLP population.

This study confirms the results of Gallardo & Castro (1987) on cross-breeding between the YAV and TEX populations, since in both cases, only 40% success was attained, similarly to the 30% success reported by these authors. Castro *et al*. (1989) reported a maximun of 60% success with the cross-breeding between SLP/TEX, similarly to the present results.

Regarding sex in cross-breeding, we observed that males showed higher reproductive isolation, particularly those of SLP followed by those of OHUI.

When populations were subjected to a second cross-breeding to obtain F_2, it was observed that some of those with 100% reproductive success in the first one, also yielded 100% success in the second, as for example in the cross-breeding between TEX females and R.SAL males. The opposite occurred with the descendants of C.CIEN females/TEX males, which depicted 100% success in the first, but 0% in the second.

The numbers of nauplii obtained through cross-breeding, in both F_1 and F_2 were reduced except for the cross-breeding of TEX female with R. SAL male, which was higher (445 and 401 for F_1 and F_2, respectively).

We can assume that the Mexican populations are not completely separated genetically, because they still produce nauplii (F_2), but they exhibit low viability. These results are similar to those of Abatzopoulos *et al.* (1998) with *A. tibetiana* and *A. franciscana.* We consider that the gradual built-up of genetic barriers has given rise to speciation in these populations, mainly in those where the ionic composition of the water is different in regard to sodium chloride, as occurs in the populations of inland waters (SLP, C.CIEN, and TEX).

Overall, we can conclude that the studied Mexican populations of *Artemia* might be in an evolutionary process of reproductive isolation, which could be attributed to the adaptation of each population to its specific habitat, which has printed these characteristics in the genotypes of these populations, causing pre- and post-mating barriers.

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