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Genetica. — *Further evidence of genetic differentiation among geographic populations of gonochoristic* Artemia (*). Nota di EMANUELE RODINÒ (**), VITTORIO VAROTTO (***), BRUNO BATTAGLIA (***) e CLAUDIO BARIGOZZI (****), presentata (*****) dal Corrisp. B. BATTAGLIA.

RIASSUNTO. — Vengono riportati i risultati di nuovi incroci eseguiti tra 5 popolazioni bisessuate di *Artemia* dalle origini seguenti: Trapani (Italia), Sfax (Tunisia), San Francisco (USA), Sonora (Messico) e Argentina (presumibilmente Hidalgo). Si è dimostrato che i due ceppi mediterranei sono geneticamente incompatibili sia tra loro che con le popolazioni americane. Questi risultati suggeriscono l'opportunità di approfondire il problema della speciazione nelle popolazioni anfigoniche di *Artemia* nel bacino mediterraneo. Nell'attesa di una loro migliore caratterizzazione non vengono proposti nomi specifici per le popolazioni di Trapani e Sfax. Per quanto riguarda le popolazioni americane, i risultati confermano che quelle di San Francisco e dell'Argentina sono ascrivibili rispettivamente alle specie *A. franciscana* e *A. persimilis*, e indicano che anche la popolazione di Sonora appartiene alla sp. *A. franciscana*. La popolazione di San Francisco si è rivelata più produttiva rispetto a quella di Sonora. Nell'incrocio tra le due sono stati messi in evidenza fenomeni di probabile eterosi.

INTRODUCTION

Since the description of *Artemia salina* as a " collective species " by Artom (1931) and the subdivision of the genus with respect to the type of reproduction into bisexual *Artemia* and parthenogenetic *Artemia* by Barigozzi (1936), much of the research carried out in the following years has been devoted to clarifying the taxonomy and modes of speciation in this genus.

Kuenen (1939) was the first Author to detect reproductive isolation between two populations of the bisexual form of *Artemia*: namely, one from California and the other from Sardinia. Later, Bowen (1965) confirmed this finding. Halfer Cervini *et al.* (1967, 1968) while comparing two populations, respectively from San Bartolomeo (Cagliari) and from Hidalgo (Argentina), found that two forms occurred together in both the populations: " form A " with 42

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chromosomes, and " form B " with 44 chromosomes. Since these two forms proved reproductively isolated, the Authors considered them sibling species.

Piccinelli and Prosdocimi (1968) described the two forms, calling the one with 42 chromosomes *Artemia salina*, and that with 44 chromosomes *Artemia persimilis*. Piccinelli, Prosdocimi, and Baratelli Zanbruni (1968) described a third species, collected from San Francisco salterns and the Great Salt Lake, for which Barigozzi (1974) proposed the name of *Artemia franciscana*.

Clark and Bowen (1976) found a new population in the salterns of Chott Ariana (Tunisia) reproductively isolated from the Hidalgo and San Francisco populations, and interfertile with " San Bartolomeo ". The Authors defined it as a new species: *Artemia tunisiana* (see also Bowen *et al.*, 1980).

Barigozzi (1980, 1982) in revising the genus, proposed that, until a thorough study of the Mediterranean populations of bisexual *Artemia* will be available, they should collectively be called *Artemia* sp.

In this paper, the results of new interpopulation crosses are reported. They provide further evidence of reproductive isolation among gonochoristic populations of *Artemia*, and examples of other kinds of genetic diversification.

MATERIALS AND METHODS

Cysts samples from five geographic populations were kindly supplied by the *Artemia* Int. Reference Center (Ghent, Belgium). The data concerning chromosome numbers were provided by one of us (Barigozzi). The stocks were the following:

— *Artemia franciscana*	(42	chroms.)	San Francisco, U.S.A.	(SFR)
— *Artemia franciscana*	(42	»)	Sonora, Mexico.	(SON)
— *Artemia persimilis*	(44	»)	Argentina.	(ARG)
— *Artemia* sp.	(42	»)	Sfax, Tunisia.	(SFA)
— *Artemia* sp.	(42	»)	Trapani, Italy.	(TRA)

All the experiments were conducted utilizing natural sea water (Salinity, 34-35‰; Temperature, 22 °C.). After hatching, groups of 50 nauplii were placed separately into cups containing 50 ml sea water, 50 ml of a *Microchlorella* culture (prepared utilizing Erdschreiber medium) and a few drops of the commercial *Artemia* nutrient " Liquizell ". Every other day 50 ml of the culture medium were replaced by an equal amount of algal suspension, until the stage of juvenile was reached (Barigozzi, 1939). At this stage each individual was transferred into single cups with 10 ml of *Microchlorella*. Also in this case the 10 ml of liquid were replaced once every two days with a corresponding amount of algal suspension. When the secondary sexual characters became manifested, a male and a virgin female of different origins (Table I) were placed together in a single cup. Thirty cups were prepared for each type of bot inter- and intra-population crosses. On deposition of each brood (nauplii and/or cysts),

each mating pair was transferred to a fresh 20 ml cup. When cysts were produced, they were at first desiccated and then allowed to hatch in sea water in order to establish the fertility of crosses. Crosses were considered fertile when the cysts so treated gave rise to nauplii which developed into normally fertile adults. In the case of infertile crosses the cysts did not produce nauplii.

In addition to the detection of possible reproductive barriers among the five populations studied, other biological parameters such as productivity, longevity, and minimum generation time, were taken into account.

This paper deals mainly with the problem of genetic compatibility of the five populations. Some data on the productivity of crosses are also provided, whereas the data on other population statistics will be the object of a further publication.

Results and Discussion

The crosses between the populations of San Francisco, Argentina, Sfax and Trapani produced only infertile cysts, with the following exceptions: the cross ♀SFA × ♂SFR gave rise to eggs which did not develop; from the reciprocal crosses between TRA × ARG, in addition to infertile cysts some nauplii were produced which, however, only survived a few days. The control crosses (intrapopulation) produced offspring which developed normally and in turn gave rise to a new generation (Table I).

Table I.

Crosses among five populations of Artemia *from different geographic localities.*

(N, normally developing nauplii; N*, abortive nauplii; E, abortive eggs; C normally hatching cysts; C*, abortive cysts; (*n*), number of mating pairs).

♀♀ \ ♂♂	SFR	SON	ARG	SFA	TRA
SFR	N+C (24)	N+C (30)	C* (30)	C* (30)	C* (20)
SON	N+C (30)	N+C (30)	—	—	—
ARG	C* (30)	—	N+C (30)	C* (20)	N*+C* (20)
SFA	E (30)	—	C* (30)	N+C (30)	C* (30)
TRA	C* (30)	—	N*+C* (35)	C* (35)	C (30)

The results concerning the American populations were, in part, to be expected on the assumption that the specimens from San Francisco and Argentina belonged to different species, namely *A. franciscana* and *A. persimilis*. Our results confirm their nature of good species. As to the specimens from Sonora (Mexico), Badaracco *et al.* (in press) have shown recently that their heterochromatin differs to a certain extent from that described in the population from San Francisco. Hence the opportunity of the crossing SON × SFR: all matings between the two populations proved fertile, indicating that both of them belong to the species *A. franciscana* (Table I). The numbers of offspring per brood, produced by the two populations, are nearly equal (Table II). However, the overall productivity of the intrapopulation cross SFR × SFR, in terms of number of broods produced, results higher compared with that observed in the cross

TABLE II.

Productivity of crosses ♀SFR × ♂SON *and respective controls.*

In crosses with ♀ SFR the figures refer to the first 4 broods produced; in crosses involving the less productive ♀ SON, to the first 3 broods. In both cases, ~ 90% of broods produced are included.

	♀ SFR × ♂ SFR				♀ SON × ♂ SON		
Brood	1°	2°	3°	4°	1°	2°	3°
No. females .	24	21	15	12	27	15	4
No. cysts + nauplii . .	852	880	771	614	942	718	178
$\bar{x} \pm$ s.d. . . .	35.50 ±11.51	41.90 ±13.38	51.40 ±17.65	51.17 ±17.64	34.89 ±13.37	47.87 ±11.17	44.50 ±25.53
Broods per female $\bar{x} \pm$ s.d.	3.37 ± 1.71				1.70 ± 0.72		
	♀ SFR × ♂ SON				♀ SON × ♂ SFR		
Brood	1°	2°	3°	4°	1°	2°	3°
No. females .	27	20	17	12	29	19	11
No. cysts + nauplii . . .	1,667	1,423	1,779	1,268	1,045	831	656
$\bar{x} \pm$ s.d. . . .	61.74 ±14.50	71.15 ±18.69	104.65 ±19.56	105.67 ±21.01	36.03 ±14.56	43.74 ±15.85	59.64 ±19.63
Broods per female $\bar{x} \pm$ s.d.	3.44 ± 2.08				2.41 ± 1.28		

TABLE III.

Statistical comparison (t-Student) of productivities within each pair of subsequent broods, and between the total numbers of broods, applied to the data reported in Table II.

Parental cross ♀ × ♂	Brood				Total number of broods
	1°	2°	3°	4°	
SFR × SFR SON × SON	n.s.	n.s.	n.s.	—	$P < 0.01$
SFR × SFR SFR × SON	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	n.s.
SON × SON SON × SFR	n.s.	n.s.	n.s.	—	$0.05 > P > 0.01$

SON × SON. The outcome of interpopulation crosses indicates the predominant effect of females in this respect. Moreover, the analysis of productivity reveals the superiority of the interpopulation crosses SFR × SON (Table III), but the outcome, presumably as a consequence of the greater contribution of females to productivity, appears to depend on the origin of the mother: In the cross ♀ SFR × ♂ SON, compared with the intrapopulation cross SFR × SFR, a significant hybrid vigour can be observed, exhibited in this case by the higher number of hybrid nauplii and/or cysts produced per brood, whereas the number of broods produced remains unchanged. In the reciprocal cross ♀ SON × × ♂ SFR, compared with the cross SON × SON, the hybrid superiority is, instead, expressed in terms of the average number of broods produced per female. In either case the constant result was that the females from both populations produced more offspring when fertilized by males of different origin. However, further research is required in order to establish the possible heterotic nature of this effect. In the marine habitat, a similar occurrence of superiority of interpopulation crosses compared with the intrapopulation controls was found in four species of the Harpacticoid Copepod *Tisbe* (Battaglia and Volkmann-Rocco, 1973).

Concerning the two Mediterranean populations, Sfax and Trapani, no previous data were available as to their genetic compatibility with other populations. The cross-breeding experiments reported in this paper show that they are reproductively isolated from the American populations as well as from

each other. These new data emphasize the complexity of the genus *Artemia* from the Mediterranean region, and suggest the opportunity of conducting further studies aiming to a better understanding of the speciation mechanisms which operate in the gonochoristic populations of *Artemia* living in that area. For the time being we shall not assign specific names to the two populations, and propose to call them respectively *Artemia* sp. Sfax and *Artemia* sp. Trapani Nubia.

REFERENCES

ARTOM C. (1931) – *L'origine e l'evoluzione della partenogenesi attraverso i differenti biotipi di una specie collettiva* (A. salina) *con speciale riferimento al biotipo diploide partenogenetico di Sete*. « Mem. R. Ital. Cl. Fis. Mat. Nat. », *2*, 1-57.

BADARACCO G., BARATELLI G., GINELLI E., MENEVERI R., PLEVANI P., VALSASNINI P. e BARIGOZZI C. (1987) – *Variation in repetitive* DNA *and heterochromatin in the Genus* Artemia. « Chromosoma », *95*, 71–75.

BARIGOZZI C. (1938) – *Anfigonia e poliploidismo in* Artemia salina. « Boll. Soc. It. Biol. Sper. », *13*, 655-656.

BARIGOZZI C. (1939) – *La biologia di* Artemia salina *Leach studiata in acquario* (*Morfologia e velocità di sviluppo*). « Atti Soc. Ital. Sci. Nat. », *78*, 137-160.

BARIGOZZI C. (1974) – Artemia: *a survey of its significance in genetic problems*. « Evolutionary Biol. », *7*, 221-252.

BARIGOZZI C. (1980) – *Genus* Artemia: *problems of systematics. The brine shrimp.*, Artemia, Vol. 1, *Morphology genetics, radiobiology, toxicology*. (Ed. by G. Persoone, P. Sorgeloos, O.A. Roels and E. Jaspers), pp. 147-153. Universa Press, Wetteren, Belgium.

BARIGOZZI C. e BARATELLI-ZAMBRUNI L. (1982) – *New data on the chromosome number of the genus* Artemia. « Rend. Acc. Naz. Lincei », *73*, 139-143.

BATTAGLIA B. and VOLKMANN-ROCCO B. (1973) – *Geographic and Reproductive isolation in the Marine Harpacticoid Copepod* Tisbe. « Marine Biology », *19*, 156-160.

BOWEN S.T. (1965) – *The genetics of* Artemia salina. VI. *Crossing over between the* X *and* Y *chromosomes*. « Genetics », *52*, 695-710.

BOWEN S.T., DAVIS M.L., FENSTER S.R. and LINDWALL G.A. (1980) – *Sibling species of* Artemia. *The brine-shrimp* Artemia, Vol. 1, *Morphology, genetics, radiobiology, toxicology*. (Ed. by G. Persoone, P. Sorgeloos, D.A. Roels and E. Jaspers), pp. 155-167.

CLARK L.S. and BOWEN S.T. (1976) – *The genetics of* Artemia salina. VII. *Reproductive isolation*. « Jour. of Hered. », *67*, 385-388.

HALFER-CERVINI A., PICCINELLI M. e PROSDOCIMI T. (1967) – *Fenomeni d'isolamento genetico in* Artemia salina. « Atti A.G.I. », *12*, 312-327.

HALFER-CERVINI A., PICCINELLI M., PROSDOCIMI T. e BARATELLI-ZAMBRUNI L. (1968) – *Sibling species in* Artemia (*Crustacea, Branchiopoda*). « Evolution », *22*, 373-381.

KUENEN D.J. (1939) – *Systematical and physiological notes on the brine shrimp*, Artemia. « Arch. Néerl. Zool. Leiden », *3*, 365-449.

PICCINELLI M. e PROSDOCIMI T. (1968) – *Descrizione tassonomica delle due specie* Artemia salina *L. e* Artemia persimilis *n.sp.* « Rend. Ist. Lomb. Sci. Lett. », *B 102*, 113-118.

PICCINELLI M., PROSDOCIMI T. e BARATELLI-ZAMBRUNI L. (1968) – *Ulteriori ricerche sull'isolamento genetico nel genere* Artemia. « Atti A.G.I. », *13*, 170-179.