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The incidence of rare males in seven parthenogenetic *Artemia* (Crustacea: Anostraca) populations

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Abstract: Males that present infrequently in parthenogenetic *Artemia* are commonly called "rare males". By rearing cloned parthenogenetic *Artemia* in the laboratory, the frequency of rare males was observed for seven *Artemia* populations with different ploidy compositions. The results showed that the values of the male:female ratio varied from 0.53:1000 to 1.40:1000 in diploids from different populations, and there were significant differences among populations and clones. No rare males were found in any of the 46 polyploid clones (217,197 individuals) observed.

Key words: Parthenogenetic *Artemia*, rare male, diploid, polyploid

Parthenogenesis is known as the development of embryos from female ovaries unfertilized with any sperm (Martens et al., 2009). Although obligatory parthenogenesis is not common in animals, the genus *Artemia* (Crustacea: Anostraca) includes a large number of parthenogenetic populations. Parthenogenetic *Artemia* consist of diploid and polyploid (i.e. tri-, tetra-, pentaploid), and also heteroploid linages (Sun et al., 1999; Abatzopoulos et al., 2002, 2003; Saygı, 2004).

In bisexual *Artemia*, the female is heterozygous (WZ) and the male is homozygous (ZZ) (Saavedra and Amat, 2005; De Vos et al., 2013), and a sex ratio of 1:1 is expected in the next generations. The reproduction of parthenogenetic *Artemia* almost invariably produces female offspring, but infrequent males (usually called "rare males") have been reported for a number of populations (e.g., Bowen et al., 1978; MacDonald and Browne, 1987; Maccari et al., 2013). Previous studies documented that the ratio of rare males varied greatly among populations (e.g., MacDonald and Browne, 1990; Qian et al., 1992; Ren et al., 1992; Cai, 1993; Maccari et al., 2013), whereas the difference in the incidence of rare males among lineages of different ploidy levels has not been experimentally studied.

By rearing cloned parthenogenetic *Artemia* (65 clones established from seven populations, representing di-, tri-, tetra-, and pentaploidy), we observed the relationships of the incidence of rare males with populations, ploidy levels, and clones.

Seven populations of parthenogenetic *Artemia*, which were collected from China and Pakistan, were used in the present study (Table 1). After resting eggs (cysts) were hatched in salinity of 32–33 psu under optimal conditions (Sorgeloos et al., 1986), and individuals were separately cultured in 50-mL Falcon tubes under standard conditions at 70 psu and 25 ± 1 °C. A mixed diet of *Dunaliella salina* and trading shrimp diet LANSY ZM (INVE, Thailand) was supplied according to the feeding schedule given by Coutteau et al. (1992). During the culture period, the reproduction mode (parthenogenesis) was confirmed, clones were established, and the ploidy level of each clone was determined by customary chromosome preparation techniques for newly produced nauplii (Abatzopoulos et al., 1986).

For each population and ploidy level, 3 to 8 clones were selected to monitor the incidence of rare males. For each clone, up to 200 nauplii were inoculated in a 10-L plastic aquarium and cultured under standard conditions. During the experiment, adults were taken out from cultures at 5- to 12-day intervals and females and potential rare males were counted. Because the Caka Lake (CK) population did not produce nauplii under the experimental conditions, resting eggs (third or fourth generation) were collected and treated at –18 °C for 30-45 days to break diapause. Nauplii that hatched from these resting eggs were reared to adulthood and females and potential rare males were counted.

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Table 1. Information of studied parthenogenetic *Artemia* populations.

Population code	Locality	Geographical coordinates
AB	Aibi Lake, Xinjiang, China	44°53′N, 82°00′E
YNG	Yinggehai Saltern, Hainan, China	18°31′N, 108°44′E
CK	Caka Lake, Qinghai, China	33°58′N, 80°53′E
DF	Dongfang Saltern, Hainan, China	19°11′N, 108°41′E
BRK	Barkol Lake, Xinjiang, China	43°40′N, 92°44′E
AQK	Aqqikkol Lake, Xinjiang, China	37°04′N, 88°22′E
KS	Karachi Saltern, Sindh, Pakistan	24°48′N, 66°85′E

The frequency of rare males was expressed as the number of rare males per 1000 females. The chi-square test, which was performed by SPSS 16, was used to determine the significant difference of the incidence of rare males among clones as well as among populations.

In all the clones observed, the occurrence of rare males was only found in diploid cultures; no males were found in any of the 46 polyploid clones (217,197 individuals) (Table 2). In five diploid clones (AB-03, AB-37, BRK-62, AQK-23, KS-04), no rare males were found (Table 2), probably

Table 2. Frequency of males and females in the studied parthenogenetic *Artemia*. For abbreviated codes for populations, see Table 1.

Population	Ploidy degree	Lab code of clones	Number of individuals	Number of males	Number of females	Male: female
AB	2n	AB-01	2455	2	2453	0.815:1000
		AB-03	1270	0	1270	0.000:1000*
		AB-05	24,882	52	24,830	2.094:1000
		AB-12	1540	1	1539	0.650:1000
		AB-29	8738	5	8733	0.573:1000
		AB-34	1090	1	1089	0.918:1000
		AB-35	3760	2	3758	0.532:1000
		AB-37	1354	0	1354	0.000:1000*
		Total	45,089	63	45,026	1.399:1000
	3n	AB-07	10,230	0	10,230	0.000:1000
		AB-21	1335	0	1335	0.000:1000
		AB-30	2755	0	2755	0.000:1000
		Total	14,320	0	14,320	0.000:1000
YNG	4n	YNG-01	12,325	0	12,325	0.000:1000
		YNG-10	7580	0	7580	0.000:1000
		YNG-15	10,125	0	10,125	0.000:1000
		Total	30,030	0	30,030	0.000:1000
	5n	YNG-11	12,760	0	12,760	0.000:1000
		YNG-17	9380	0	9380	0.000:1000
		YNG-25	6875	0	6875	0.000:1000
		YNG-49	11,690	0	11,690	0.000:1000
		Total	40,705	0	40,705	0.000:1000

Table 2. (Continued).

Population	Ploidy degree	Lab code of clones	Number of individuals	Number of males	Number of females	Male: female
CK	3n	CK-07	7934	0	7934	0.000:1000
		CK-12	2468	0	2468	0.000:1000
		CK-19	3792	0	3792	0.000:1000
		Total	14,194	0	14,194	0.000:1000
	4n	CK-02	5560	0	5560	0.000:1000
		CK-08	1674	0	1674	0.000:1000
		CK-24	2161	0	2161	0.000:1000
		Total	9395	0	9395	0.000:1000
DF	3n	DF-01	3856	0	3856	0.000:1000
		DF-09	1745	0	1745	0.000:1000
		DF-26	1930	0	1930	0.000:1000
		Total	7531	0	7531	0.000:1000
	4n	DF-03	3160	0	3160	0.000:1000
		DF-06	2215	0	2215	0.000:1000
		DF-45	1640	0	1640	0.000:1000
		Total	7015	0	7015	0.000:1000
	5n	DF-02	2050	0	2050	0.000:1000
		DF-10	1185	0	1185	0.000:1000
		DF-48	1065	0	1065	0.000:1000
		Total	4300	0	4300	0.000:1000
BRK	2n	BRK-53	24,750	15	24,735	0.606:1000
		BRK-58	5270	3	5267	0.570:1000
		BRK-62	3682	0	3682	0.000:1000*
		BRK-86	4113	2	4111	0.486:1000
		Total	37,815	20	37,795	0.529:1000
	3n	BRK-25	7935	0	7935	0.000:1000
		BRK-70	2864	0	2864	0.000:1000
		BRK-82	3411	0	3411	0.000:1000
		Total	14,210	0	14,210	0.000:1000
	4n	BRK-16	8472	0	8472	0.000:1000
		BRK-28	2275	0	2275	0.000:1000
		BRK-92	3565	0	3565	0.000:1000
		Total	14,312	0	14,312	0.000:1000
	5n	BRK-22	6280	0	6280	0.000:1000
		BRK-45	1870	0	1870	0.000:1000
		BRK-116	3290	0	3290	0.000:1000
		Total	11,440	0	11,440	0.000:1000

Table 2. (Continued).

Population	Ploidy degree	Lab code of clones	Number of individuals	Number of males	Number of females	Male: female
AQK	2n	AQK-07	23,675	27	23,653	1.142:1000
		AQK-14	4820	3	4817	0.623:1000
		AQK-23	1785	0	1785	0.000:1000*
		AQK-35	3810	2	3808	0.525:1000
		Total	34,090	32	34,063	0.939:1000
	3n	AQK-02	14,930	0	14,930	0.000:1000
		AQK-12	1854	0	1854	0.000:1000
		AQK-40	2781	0	2781	0.000:1000
		Total	19,565	0	19,565	0.000:1000
	4n	AQK-01	11,665	0	11,665	0.000:1000
		AQK-17	1215	0	1215	0.000:1000
		AQK-27	2580	0	2580	0.000:1000
		Total	15,460	0	15,460	0.000:1000
KS	2n	KS-01	18,234	12	18,222	0.659:1000
		KS-04	2855	0	2855	0.000:1000*
		KS-08	3725	3	3722	0.806:1000
		Total	24,814	15	24,799	0.605:1000
	3n	KS-03	5120	0	5120	0.000:1000
		KS-07	1280	0	1280	0.000:1000
		KS-32	1040	0	1040	0.000:1000
		Total	7440	0	7440	0.000:1000
	4n	KS-02	4660	0	4660	0.000:1000
		KS-11	1450	0	1450	0.000:1000
		KS-28	1170	0	1170	0.000:1000
		Total	7280	0	7280	0.000:1000

*Not used in the chi-square test for the diploid clones from the same population (see Table 3).

because only small numbers of adults were observed for these clones.

The chi-square test showed that the incidence of rare males was significantly different among diploids from four populations (AB, BRK, AQ, and KS) (Table 3). Significant difference was also detected among diploid clones of Aibi Lake (Table 3).

Previous studies have documented extensive interpopulation variation of sex ratios among different populations of parthenogenetic *Artemia* (Bowen et al., 1978; MacDonald and Browne, 1987, 1990; Qian et al., 1992; Ren et al., 1992; Cai, 1993; Asem et al., 2010; Maccari et al., 2013), whereas the ploidy levels were not considered in these studies. The male:female ratio recorded for wild "parthenogenetic" populations varied from 1:6086 to 1:67 (MacDonald and Browne, 1990; Ren et al., 1992). For cultured populations, this ratio was reported to be from 1:12,499 (Gerri, Spain) (Maccari et al., 2013) to 1:11 (493 males:5595 females; recorded for a selected clone from China) (Cai, 1993). The present results show that there is significant difference among the sex ratios of the diploid *Artemia* from four populations with the values of male:female varying from 0.529:1000 to 1.399:1000 (Tables 2 and 3). Significant difference was also detected among different clones of the same population (Tables 2 and 3). Such intrapopulation difference in rare male frequency was

Table 3. The results of the chi-square test for the sex ratio (male:female) among different diploid *Artemia* clones of the same population, and among different populations. For abbreviated codes for populations, see Table 1.

Populations (diploid)	χ^2	df	P
AB	15.07	5	0.010
BRK	0.09	2	0.956
AQ	2.03	2	0.362
KS	0.10	1	0.754
Among different populations ("Total" of diploids shown in Table 2)	20.23	3	0.000

also reported for the Salin de Giraud population (France) (MacDonald and Browne, 1990). Therefore, the existing data show that the frequency of rare males in diploid parthenogens are, at least to some extent, determined by their genetic makeup.

Among the seven studied populations, rare male ratios have been formerly reported for the DF, AB, and BRK populations. Although the male:female ratio in cultured samples of the DF population was reported as 1:637 by Maccari et al. (2013), we could not clone diploid females in this population. High variable male:female ratios have been reported for the AB population. For instance, Ren et al. (1992) documented the ratio in a wild sample as 1:6086 (15 males in 91,311 specimens), Qian et al. (1992) reported a ratio of 1:625 (whether from a wild or a laboratory population is unknown), and Maccari et al. (2013) observed a much higher ratio of 1:116 (19 males in 2207 specimens) from cultured samples. Moreover, Qian et al. (1992) documented the male:female ratio of the BRK population as 1:189. The difference in the ploidy composition of samples used by different authors could be a reason for the significant variation of the rare male ratio of the same population. However, this cannot explain the higher rare male ratios observed by Qian et al. (1992) and Maccari et al. (2013) for the BRK and AB populations. Some unknown factors may impact the incidence of rare males in parthenogenetic *Artemia*. Environmental conditions are well-known factors to induce the production of males in cyclical parthenogenesis (e.g., rotifers). However, their effects on the occurrence of rare males in *Artemia* remain still to be studied.

No rare males were found in any of the 46 polyploid clones (217,197 individuals) from seven populations (Table 2), and they were not recorded in any previous studies. Diploids and not polyploids being able to produce rare males might be because diploid parthenogenetic *Artemia* are automictic while polyploids are apomictic (Barigozzi, 1974; Browne and Bowen, 1991; Triantaphyllidis et al., 1998; Abatzopoulos et al., 2002, 2003).

In conclusion, rare males of parthenogenetic *Artemia* occur only in diploids. Their frequencies may be significantly variable among populations, as well as among diploid strains from the same population. Genetic makeup should be a factor determining the incidence of rare males in diploid parthenogenetic *Artemia*.

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