FIRST REPORT OF GYNANDROMORPHY IN PARTHENOGENETIC *ARTEMIA* (BRANCHIOPODA: ANOSTRACA)

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A B S T R A C T

We describe three diploid gynandromorphs in *Artemia*. Two belong to *A. parthenogenetica* and represent the first report of gynandromorphy for the species. One specimen was a female with a globular brood-pouch and gynandromorphic second antennae, whilst the other was a female with a closed malformed brood-pouch filled with live nauplii, and a long, mobile, penis-like structure projecting from the left side of the genital segments. Offspring from the latter parthenogenetic gynandromorph developed as normal all-female parthenogenetic *Artemia*. The third gynandromorph belongs to the zygogenetic species *A. franciscana*, being a female with claspers as previously reported in the same taxon. The occurrence of parthenogenetic gynandromorphs is best explained in light of sex determination mechanisms supporting the view that gynandromorphy might be triggered by a mitotic chromosomal mutation during early embryonic development.

INTRODUCTION

Recently, we found commercial Great Salt Lake cyst lots containing a mixture of parthenogenetic and zygogenetic species of *Artemia*, in which the origin of the former is still unknown (Campos-Ramos et al., 2003). A careful examination of parthenogenetic offspring revealed a naturally occurring female with gynandromorphic second antennae, and another with a malformed brood-pouch and a mobile penis-like structure. In addition, a gynandromorphic specimen of the zygogenetic species *A. franciscana* was found with the male second antennae and a female brood-pouch. In this work, we describe the morphology of these unique animals, and discuss relevant models of the origin of gynandromorphy in brine shrimp.

A gynandromorph is an individual exhibiting a spatial mosaic of female and male characteristics. Gynandromorphism rarely occurs in the crustacean anostracans. Theoretically, gynandromorphs arise from early embryonic mitotic aberrations in brine shrimp (Bowen and Hanson, 1962; Bowen et al., 1966) or under the influence of epigenetic factors on segmental gender differentiation during larval development in *Branchinecta*, without discarding mitotic aberrations (Sassaman and Fugate, 1997). The review on gynandromorphs in Anostraca by Sassaman and Fugate (1997) does not report gynandromorphic specimens from parthenogenetic brine shrimp females.

The occurrence of gynandromorphism in brine shrimp with sexual reproduction, such as the zygogenetic *Artemia franciscana* Kellogg, 1906, has provided a wide morphological description of sexual mosaics, and discussions on the origin of gynandromorphism in this type of animal (Bowen et al., 1966; Sassaman and Fugate, 1997). However, finding a sexual mosaic in diploid brine shrimp *Artemia parthenogenetica* Bowen and Sterling, 1978 is intriguing because parthenogenetic females reproduce asexually and all progeny are expected to be composed of parthenogenetic females. Occasionally though, sexually active males are produced (MacDonald and Browne, 1987, 1989; Gao et al., 1995; Campos-Ramos et al., 2003). We think that an explanation of how a sexual mosaic arose from a parthenogenetic individual should depart from an explanation of the sex determination mechanism and the presence of sex chromosomes. Thus, we address the following questions; How do parthenogenetic females produce all-female progeny after restoring diploidy? Should sex chromosomes recombine at meiosis? How does a male suddenly appear in the progeny? Are sexual steroids or sex-diffusible substances involved in the process of sex differentiation?

MATERIALS AND METHODS

Parthenogenetic and zygogenetic *Artemia* cysts were obtained from commercial cans from the Great Salt Lake. Hatching, culture, and breeding of brine shrimp are explained in Campos-Ramos et al. (2003). Animals were anesthetized in seawater with carbonated water (5:1) prior to examination and photography. The specimens were deposited in the Crustacea collection at the Centro de Investigaciones Biológicas del Noroeste, S. C., La Paz, Baja California Sur, México. A video-CD of the malformed parthenogenetic *Artemia* is available from the corresponding author on request.

RESULTS

Artemia parthenogenetica Gynandromorph

The specimen was bright red, and had long antennules (first antennae), abdomen, and cercopods (Fig. 1A). These basic morphological traits distinguish parthenogenetic *Artemia* from females of *A. franciscana* (see Campos-Ramos et al., 2003). The genitalia had a globular brood-pouch with two protuberances around the opening (Fig. 1B). The left second antenna looked like a normal parthenogenetic male second antenna and was composed of a semi-rectangular distal joint and a basal joint with the typical knob (Fig. 1C, D). The right second antenna was reduced and looked like a normal parthenogenetic female second antenna. It was composed of a triangular apical joint and a basal joint without a knob (Fig. 1D).
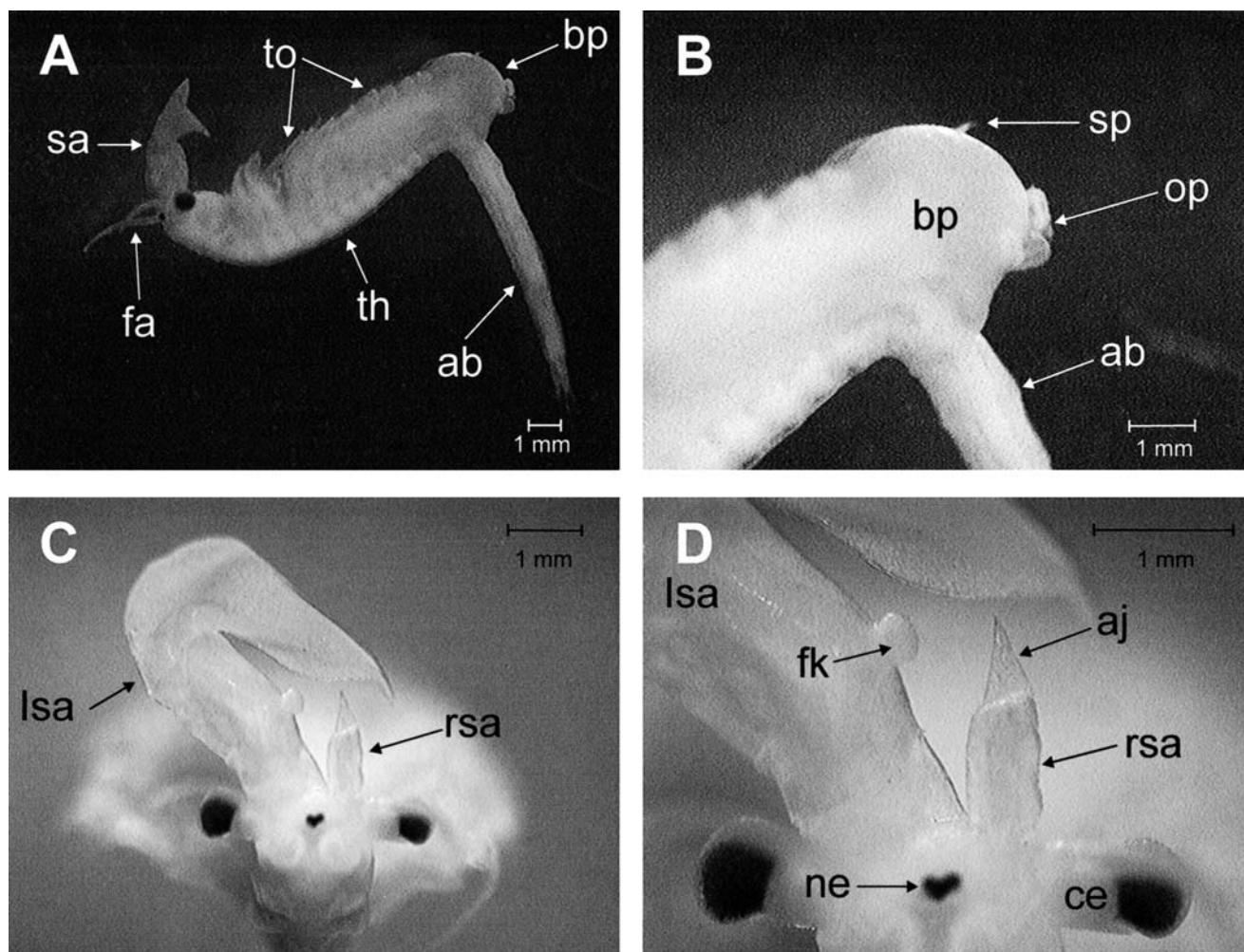
Fig. 1.   A-D: *Artemia parthenogenetica* gynandromorph. A, Right lateral view of complete specimen; B, Right lateral view of genitalia; C, Anterior view of head showing the second antennae; D, Detailed view from C. ab = abdomen, aj = apical joint, bp = brood-pouch, ce = compound eye, fa = first antennae, fk = frontal knob, lsa = left second antenna, ne = nauplius eye, op = opening of brood-pouch, rsa = right second antenna, sa = second antennae, sp = spine of the brood-pouch, th = thorax, to = thoracopods.

Artemia parthenogenetica Malformed Gynandromorph

The genitalia of this specimen consisted of a closed brood-pouch with a long sharp protuberance and a conspicuous, long, mobile penis-like structure projecting from the left side of the genital segments, which protrusion was a little longer than the length of the first abdominal segment of the animal (Fig. 2A). The single penis-like structure showed a tubular form ending with a hook, moved vertically, and bent upwards in the middle of the structure (Fig. 2B, C). It was covered with a chitinous membrane similar to that of the brood-pouch. The closed brood-pouch had eggs (Fig. 2B), which later developed into nauplii (Fig. 2D, E). The specimen appeared to have functional ovaries, showing a heavy accumulation of oocytes in abdominal segments 1-3 (Fig. 2D). We carefully opened the brood-pouch and freed 150 swimming nauplii. These were reared to adults in the laboratory and were found to exhibit normal parthenogenetic female morphology. A ventral view of normal parthenogenetic female genitalia, showing a normal brood-pouch with eggs, is shown in Fig. 2F for comparative purposes.

Artemia franciscana Gynandromorph

The specimen had a normal brood-pouch containing cysts (Fig. 3A, B). The left and right second antennae were male in appearance but not fully developed. They consisted of a semitriangular distal joint and a small basal joint, and the typical knobs were present on both antennae (Fig. 3C, D).

DISCUSSION

Because the female of *Artemia* is the heterogametic sex (Bowen, 1963, 1965; Barigozzi, 1974), we denote the sex-determination system as WZ/ZZ, in place of the XX/XY system used in *Artemia* literature during the 1960s. Bowen et al. (1966) suggested that the female phenotype would arise from a female determining-locus or loci on the W-chromosome or by a balance between two homologous autosomes; WZ-chromosomes bearing W-female characteristics and ZZ-chromosomes bearing male characteristics.

In diploid parthenogenetic reproduction, there are two models of the diploidy restoration; automixis, and the fusion of spindles. Stefani (1964) suggested that restoring diploidy
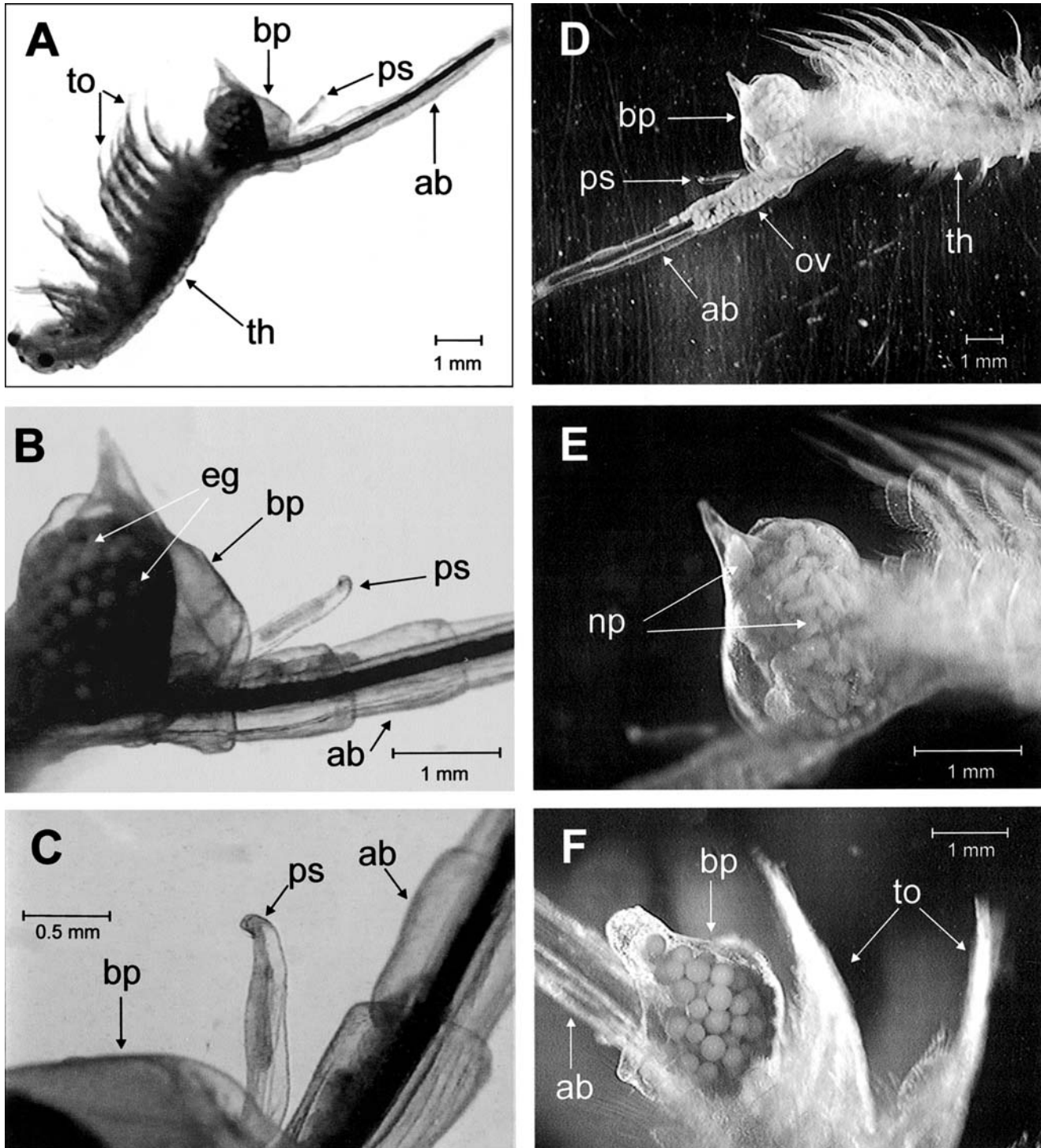
Fig. 2. A-E: *Artemia parthenogenetica* malformed gynandromorph. F: *A. parthenogenetica* normal female. A, Right lateral view of complete specimen; B, Right lateral view of genitalia showing the abnormal brood-pouch with eggs, and the penis-like structure; C, Detailed view of penis-like structure from B; D, Left lateral view of thorax, genitalia, and abdomen; E, Left lateral view of genitalia showing the abnormal brood-pouch with live nauplii; F, Ventral view of genitalia showing a normal brood-pouch with eggs. ab = abdomen, bp = brood-pouch, eg = eggs, np = nauplii, ov = ovary, ps = penis-like structure, th = thorax, to = thoracopods.

could occur through the fusion of nuclei (automixis) after the second meiotic division. We do not support this model because it implies that after meiosis four haploid cells would be present (two W-type cells and two Z-type cells). Subsequently, a random fusion of nuclei (from sister and non-sister chromatids) would give rise to ¼ of "super female" WW-type, ¼ of male ZZ-type, and ½ of female heterozygous WZ-type. This means that the occurrence of a ZZ-male would have a probability of 0.25, but this sex ratio has never been observed in parthenogenetic *Artemia*.
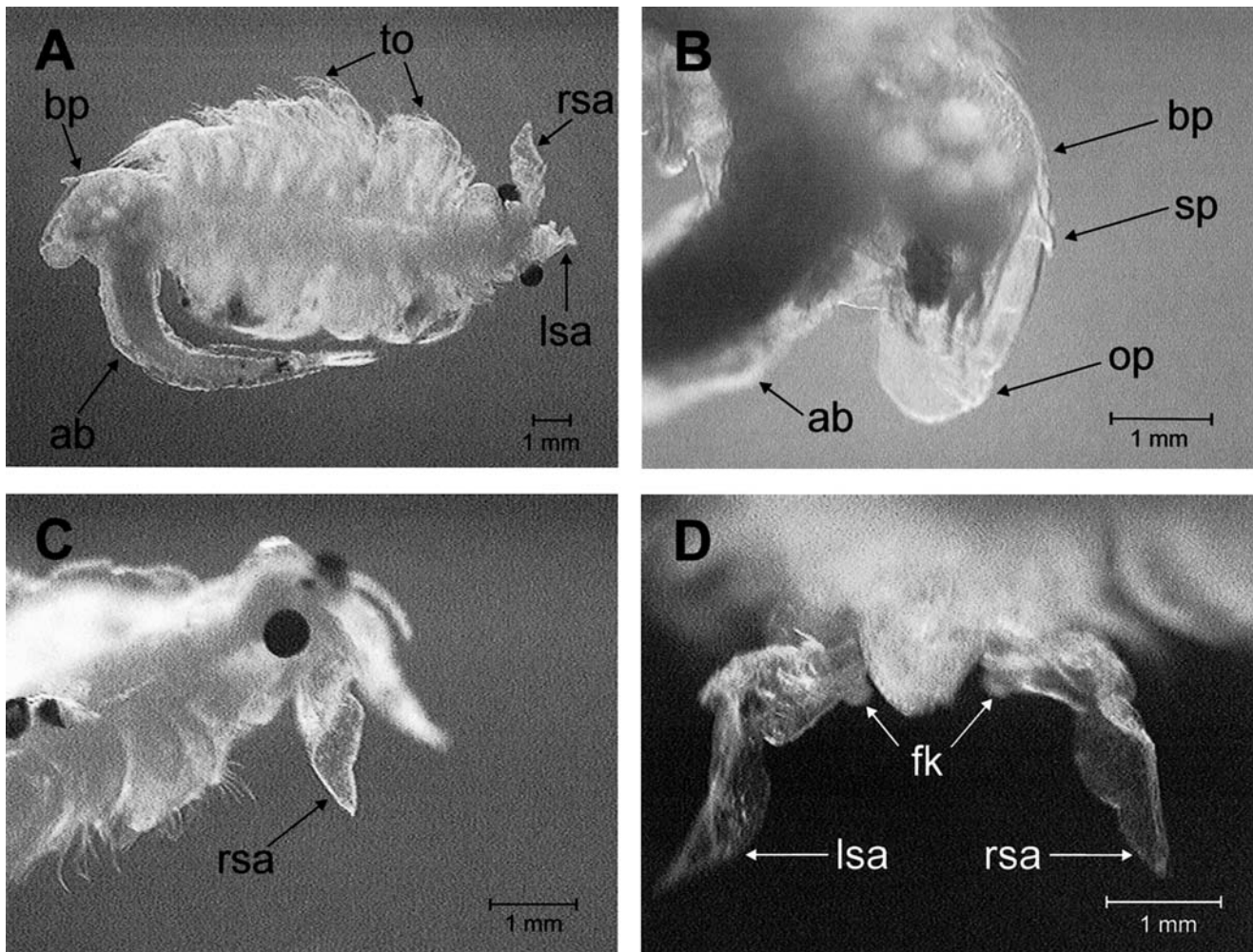
Fig. 3. A-D: *Artemia franciscana* gynandromorph. A, Ventral view of complete specimen; B, Right lateral view of genitalia; C, Right lateral view of head showing the right second antenna; D, Ventral view of second antennae. ab = abdomen, bp = brood-pouch, fk = frontal knob, lsa = left second antenna, rsa = right second antenna, sp = spine of the brood-pouch, op = opening of brood-pouch, to = thoracopods.

Barigozzi (1989) suggested that because homologous sex chromosomes generally fuse, the mechanism acted almost exclusively in favor of the female WZ-type, eliminating WW- and ZZ-genotypes, although on rare occasions a ZZ-male could succeed. However, there has been no explanation on what genetic basis WW- and ZZ-genotypes should be eliminated, nor whether a ZZ-genotype would succeed occasionally under the premise of random fusion.

Based on cytogenetic observations, Stefani (1967) proposed that diploidy was restored during the metaphase-anaphase of the second meiotic division through longitudinal fusion of the spindles. We support Stefani's (1967) proposal because this condition implies a high recombination rate among nonsister chromatids of sex bivalents during the pachytene stage of the first meiotic division. In this way, a recombinant WZ appears at the second meiotic division, which is arrested during anaphase II and yields a diploid parthenogenetic female. If little or no recombination occurred, the sex ratio would tend to a 1:1 proportion of homozygous female WW-type and male ZZ-type, a sex ratio not observed in parthenogenetic reproduction. Bowen's

observations (1963, 1965) of low recombination rates between sex chromosomes involving the white-eye locus in zygogenetic brine shrimp suggest a distant location of sex locus or sex loci in the W-chromosome and thus support a high probability of recombination. Abreu-Grobois and Beardmore (2001) showed evidence that progeny from diploid parthenogenetic brine shrimp have some heterozygosis, which also suggests that recombination events occur during the first meiotic division.

How does a Rare Male Suddenly Appear in the Progeny?

According to Macdonald and Browne (1987), there is a constant rate of rare male production from single parthenogenetic dams, but the rate of male production varies widely among parthenogenetic females. Therefore, it appears that the production of a rare male is susceptible to selection (Macdonald and Browne, 1987) and genetically based (Abreu-Grobois and Beardmore, 2001). We propose that a single cross-over among sex chromatids does not include the sex locus (loci) and eventually produces a rare

nonrecombinant ZZ-male. Alternatively, a kind of synaptic error preventing recombination could occur eventually during zygotene and pachytene. Such an "error" could arise after an incomplete synapsis or during the formation of a recombinant nodule. Our proposal is that by either a rare exclusion of sex loci during recombination, or a rare error during synapsis, this nonrecombinant event would also produce a rare "super female" (WW), which in theory will never produce males in their linage. Therefore, it is assumed that because a ZZ-male genotype is occasionally formed, a WW-female genotype should also rarely exist and there is no reason to believe that either genotype creates a lethal condition as suggested by Barigozzi (1989). The WW-female genotype would be indistinguishable from hundreds of parthenogenetic females that would not necessarily produce a male in every reproductive cycle.

The Origin of Gynandromorphism in *Artemia*

Bowen and Hanson (1962) first observed a gynandromorphic individual of zygogenetic *A. franciscana* in the fourth generation among mating siblings derived from X-ray-irradiated progenitors. They suggested that the occurrence of a perfect bilateral gynandromorph with the right side male and testes containing sperm, and the left side female with the ovary producing yolky eggs, was caused by a possible mitotic chromosomal loss during the first cleavage. Therefore, an early embryonic mitotic aberration, such as a nondisjunction of the W-chromosome or a W-chromosomal loss, would have left an embryonic cell devoid of the female chromosome (0Z-genotype) and free to express a male tissue immersed in a female's body. At the first cleavage, such a chromosomal mutation would yield a perfect bilateral gynandromorph. In further embryonic divisions, it would yield a variable degree of spatial female and male tissue. Bowen et al. (1966) observed three perfect bilateral gynandromorphs and eight sex mosaics. The latter were internally female or male; externally they had structures differentiated into female and male as spatial sex mosaics.

From our survey of the literature, it appears gynandromorphy in parthenogenetic *Artemia* is reported here for the first time. The two parthenogenetic and the bisexual gynandromorphs described in this work had morphological features similar to those previously reported by Bowen et al. (1966) and Barigozzi (1974). The occurrence of parthenogenetic gynandromorphs is best explained in light of the sex determination mechanisms, the high recombination rate of sex chromosomes, the origin of rare nonrecombinant ZZ-males, and the restoration of diploidy (Stefani, 1967). These sex mechanisms support the idea that a faithful WZ-female clone arises from a parthenogenetic dam, which further could present a chromosomal loss during embryonic development, giving rise to a WZ-0Z spatial mosaic gynandromorph. Our parthenogenetic evidence reinforces the original proposal by Bowen and Hanson (1962) and Bowen et al. (1966).

Are Sexual Steroids Involved in the Process of Sex Differentiation?

Bowen et al. (1966) mentioned that the presence of a small amount of tissue characteristic of one sex (e.g. male antennae) in an animal composed mostly of the other sex, e.g., female body, can be interpreted as evidence that each cell in a sex-mosaic individual determines its sex autonomously, accordingly to its sex genotype. The authors also suggested that if there were sexual steroids or sex-diffusible substances involved in the process of sex differentiation, these failed to suppress the differentiation of cells carrying an opposite-sex genotype, i.e., to suppress aberrant 0Z-genotype cells involved in the formation of the male antennae by WZ-female sex compounds. If sex is determined autonomously in each cell without sex-hormone mediation (Bowen et al., 1966), male structures on a female body should develop completely. It would be difficult to explain an undeveloped structure by mitotic chromosomal aberration only. Most of the sex mosaics described by Bowen et al. (1966) accomplished the normal development of structures such as male antennae. However, some of them had an intermediate sex-structure, and some had an undeveloped or malformed structure. Our first parthenogenetic specimen showed complete development of the left second male antenna, which agrees with Bowen et al. (1966). Our second parthenogenetic gynandromorph carrying a long, mobile penis-like structure appeared possibly to be a unique individual, and a result of chromosomal mutation, combined with aberrant embryonic development. The viable nauplii from this parthenogenetic specimen yielded normal adult all-female parthenogenetic offspring of *Artemia*. This suggests that this sex mosaic had an ovary and a viable brood-pouch formed of normal WZ-female tissue, which also agrees with Bowen et al. (1966). However, in the *A. franciscana* gynandromorph, the male antennae were not completely developed in the female's body. If the antennae were composed of mutated 0Z-male cells, why did they not develop normally? A possible interpretation is that female sexual compounds affected the development of the autonomous aberrant male genotype cells, without overruling their male sex-chromosome information. Gynandromorphs are common in insects caused by cytogenetic aberrations during embryogenesis. De Loof and Huybrechts (1998) proposed that sex steroids are present in insects. Should we expect sex steroids or similar components in *Artemia* as well?

As a major conclusion, we support the proposal of Bowen et al. (1966) that mitotic mutations produce sex mosaics in brine shrimp. However, it will be necessary to analyze gynandromorphic female and male cells genetically to make a forceful conclusion. It may also be reasonable to propose that although sex differentiation takes place autonomously in each cell, the degree of development of a sex-phenotypic structure in a gynandromorph could be somewhat affected by sexual compounds.

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