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The intron 2 of p26 gene: a novel genetic marker for discriminating the two most commercially important *Artemia franciscana* subspecies

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Artemia franciscana is considered as a "superspecies", since its populations exhibit the highest level of phenotypic plasticity among *Artemia* species, display high inter-population genetic divergence and/or significant genetic sub-structuring and occur in a broad repertoire of habitats. Encysted embryos originating from two of the most abundant populations (Great Salt Lake – GSL, Utah, USA and San Francisco Bay – SFB, USA) are extensively used in aquaculture industry; these two populations are considered as two subspecies. In this study, we present a novel genetic marker (the second intron of p26 gene) for discriminating GSL and SFB populations. The evaluation of the utility of this marker was achieved by PCR amplification (51 individuals from GSL and 48 individuals from SFB) and sequencing while its mode of inheritance was assessed by 20 reciprocal cross-breeding tests. Results showed that SFB *A. franciscana* population presented only one pattern (length of PCR amplified fragment was ~1500 bp) while the GSL population showed two patterns; the first showed only one PCR amplified fragment (~2000 bp), while the second presented two bands (at ~2000 bp and ~1500 bp). BLAST searches confirmed the identity of the obtained sequences while the alignment revealed that the observed length variation was mainly attributed to a single insertion of ~400 bp long. The intron 2 of p26 gene follows the Mendelian mode of inheritance, since the expected ratios do not differ significantly from the observed ones (χ^2 test, $p > 0.05$). The proposed marker was found to be an effective and practical tool (in terms of high speed, reduced cost and low complexity) to discriminate the two *A. franciscana* populations/subspecies (GSL and SFB). This marker may be applied in aquaculture industry, molecular ecology and/or other fields of applied research.

Key words: San Francisco Bay, Great Salt Lake, superspecies, genetic differentiation, mode of inheritance, intron.

INTRODUCTION

Artemia is extensively used in aquaculture, in aquarium and pet trade, in the salt production and as a "model organism" by physiologists (e.g. Clegg, 2005), geneticists (e.g. Baxevanis *et al.*, 2006), ecotoxicologists (e.g. Nunes *et al.*, 2006; Aligizaki *et al.*, 2008) and radiobiologists (Dhont & Sorgeloos, 2002). In the 1950s, commercial sources of *Artemia* cysts initially originated from: i) the coastal saltworks in the San Francisco Bay (SFB), California, USA (37˚27′ N, 122˚16′ W) and ii) an inland lake, the Great Salt Lake (GSL), Utah, USA (41˚05′ N, 112˚30′ W). Since then, cyst prices have been fluctuating depending on the demand and the harvests from the Great Salt Lake (Lavens & Sorgeloos, 2000; Dhont & Sorgeloos, 2002). By the end of the last century, *Artemia* cysts were also commercially available from other sites/sources, mainly in America and Asia (e.g. Brazil, Iran, and PR China). Unfortunately, the quality and hatching characteristics of these cysts do not meet the standards set by the aquaculture industry and, therefore, the majority of all marketed cysts still originate from the

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Great Salt Lake (Van Stappen, 1996; Lavens & Sorgeloos, 2000).

Artemia franciscana is one of the endemic *Artemia* species found in the New World (the other one is *A. persimilis*) and it is regarded as a "superspecies" (Abatzopoulos *et al*., 2002). Conspecific populations of *A. franciscana* are distinguished from the other bisexual *Artemia* species by higher levels of phenotypic plasticity (Browne & Wanigasekera, 2000; Mura *et al*., 2006 and references therein) and inter-population divergence (Gajardo *et al*., 2002) along with significant sub-structuring (Gajardo *et al*., 1995). They also occur in habitats with the largest diversity in water chemistry (Bowen *et al.*, 1985, 1988). Furthermore, *A. franciscana* shows the highest temperature tolerance compared with *A. salina*, *A. persimilis* and parthenogenetic strains, in terms of hatchability of cysts, resistance of larvae, and biomass production (Vanhaecke & Sorgeloos, 1989; Triantaphyllidis *et al.*, 1994; Baxevanis & Abatzopoulos, 2004). Also, *A. franciscana* is more euryhaline and eurythermal when compared with the rest of the *Artemia* species and parthenogenetic strains (Triantaphyllidis *et al*., 1995). These unique features enable *A. franciscana* to colonize various environments and outcompete quickly native *Artemia* populations (Van Stappen, 2002). The invasive character of *A. franciscana* and the establishment of non-indigenous populations all over the world have been central topics of many studies (Amat *et al.*, 2005; Green *et al.*, 2005; Abatzopoulos *et al.*, 2006; Mura *et al.*, 2006; Ruebhart *et al.*, 2008).

Apart from this, *A. franciscana* can be easily discriminated from all other *Artemia* species by using morphometrical characters (Triantaphyllidis *et al.*, 1995; Baxevanis *et al*., 2005; Mura *et al.*, 2006) and molecular markers (i.e. allozymes, 16S, COI, ITS1) through a variety of techniques (RFLPs, RAPDs, AFLPs, sequencing) (Badaracco *et al.*, 1995; Sun *et al*., 1999; Abatzopoulos *et al.*, 2002; Bossier *et al.*, 2004; Gajardo *et al.*, 2004; Baxevanis *et al*., 2005, 2006; Kappas *et al.*, 2009).

Many studies on *A. franciscana* populations have revealed different intra-specific characteristics. In particular, *A. franciscana* from SFB and *A. franciscana* from GSL show strain-specificity in diapause deactivation and in hatching characteristics (Lavens & Sorgeloos, 1987). For example, a simple dehydration in brine for 24 hrs results in maximal hatching (> 90%) for SFB *Artemia* cysts whereas cysts from GSL need more sophisticated deactivation techniques, such as hibernation and peroxide treatment, to finally break diapause (Lavens & Sorgeloos, 1987). Temperature has also an important impact on cyst hatchability and biomass production, with *Artemia* from SFB being more tolerant than *Artemia* from GSL (Vanhaecke & Sorgeloos, 1989). Such differences between strains might be strongly correlated to their environment and/or genotypic differences (Lavens *et al.*, 1986; Lavens & Sorgeloos, 1987; Vanhaecke & Sorgeloos, 1989). In addition, evidence from morphological, karyological, electrophoretic, and DNA sequencing data has shown significant sub-structuring and differentiation within *A. franciscana* (Gajardo *et al.*, 2002). There are only a few studies that can discriminate these two populations. For instance, Badaracco *et al.* (1995) found that populations of *A. franciscana* show high polymorphism in RAPD markers. Moreover, Baxevanis *et al.* (2006) could discriminate SFB individuals from GSL ones by comparing their ITS1 sequences. However, both techniques are expensive, time-consuming, and require the construction of an extended database.

In the last two decades, introns have been widely used in molecular and phylogenetic analyses because they possess a number of traits that allow them to be considered as neutral (Creer, 2007). Introns are non-coding gene regions that are removed from the initial RNA transcript by splicing during maturation when functional RNA molecules are produced. They have been shown to influence expression of eukaryotic genes in many ways, such as translation and decay of the mRNA product, exon shuffling and alternative splicing (Creer, 2007 and references therein). Despite their indisputable functional significance, neutrality of introns is well-accepted among molecular phylogeneticists (Côrte-Real *et al*., 1994), since their non-coding nature anticipates the gaining of a large number of independent parsimony informative characters from most sites equally, along with less homoplasy and lower transition/transversion ratios (Creer, 2007 and references therein). However, it should be noted that some introns appear to evolve under complex and different evolutionary constraints because of their engagement in splicing mechanisms (i.e. they may possess conserved and secondary structure elements and/or mutational hotspots).

The p26 protein is a low-molecular-mass molecule that belongs to the small heat-shock/a-crystallin family as it contains a region which is significantly similar to the conserved a-crystallin domain (Liang *et al.*, 1997; Tanguay *et al.*, 2004). This protein is produced by encysted embryos of *Artemia* (although low levels

of p26 gene transcripts have been identified in ovoviviparous embryos) and acts as a molecular chaperone (Abatzopoulos *et al*., 1994) preventing protein unfolding as well as aggregation and inhibiting apoptosis (Liang & MacRae, 1999; Qiu *et al.*, 2006). The full sequence of p26 gene from *A. franciscana* and details on its structure and domains has revealed that p26 is well-conserved across *Artemia* species and contains three introns (Qiu *et al*., 2006 and references therein).

The present study is designed to investigate a novel genetic marker (second intron of the p26 gene) that could be used as a diagnostic tool for discriminating SFB and GSL *Artemia* populations (for basic and applied research). Its utility as a population-specific marker was evaluated by sequencing and cross-breeding tests.

MATERIALS AND METHODS

Populations investigated

Two *A. franciscana* populations were used: i) San Francisco Bay – SFB (*Artemia* Reference Center code no. 1258), and ii) Great Salt Lake – GSL (*Artemia* Reference Center code no. 1286).

Hatching and culture conditions

Cysts (1.6 g of dried cysts per liter of hatching medium) from these two populations were incubated in aerated conical tubes containing 50 ml of filtered artificial 35 g l^{-1} seawater (Instant Ocean). Na_2CO_3 was added to increase the pH to 8.75 and illumination was at least 2000 lux (Triantaphyllidis *et al.*, 1994). After 48 hrs of cyst incubation, hatched nauplii were transferred to 1 L cylindroconical glass tubes at an initial density of 2 nauplii ml^{-1} of culture medium and mild aeration was applied from the bottom of the tubes. The tubes were covered in order to minimize evaporation. The salinity was 55 g l^{-1} and the temperature 25 ± 1 °C (Triantaphyllidis *et al.*, 1994; Abatzopoulos *et al.*, 2002). Both conditions were kept constant throughout the culturing period and the breeding tests. The animals were fed on a mixed diet of *Nannocloropsis* and the yeast-based formulated feed LANSY PZ (INVE Aquaculture SA, Belgium) following the feeding schedule of Triantaphyllidis *et al.* (1995). Approximately 50% of the culture medium was replaced every seven days in the stock cultures and every four days for the breeding tests (see below) in the 50 ml Falcon tubes (Abatzopoulos *et al.*, 2003). The density of the animals was reduced after day 8 to one individual per 4 ml. The photoperiod was 12 hrs light/12 dark and was provided by fluorescent light tubes.

Breeding tests

A total of 20 single-pair reciprocal interpopulation crosses of adult GSL and SFB *Artemia* individuals were performed in order to evaluate the mode of inheritance of the intron 2 of p26 gene. Initially, virgin females were isolated from stock cultures before they reached first ovulation and kept for at least 14 days to ensure non-impregnation, whereas males were collected directly from stock cultures (Abatzopoulos *et al*., 2002). Pairs were transferred into separate 50 ml Falcon tubes and kept under standard culture conditions. Nauplii (F_1) were transferred into separate bottles and kept under standard culture conditions until adulthood.

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from randomly chosen adults derived from the stock cultures of SFB (48 individuals) and GSL (51 individuals) as well as from parental and F_1 individuals using 150-200 µl of Chelex suspension (6%, Bio-Rad Laboratories, CA, USA) with proteinase K (15 µl of 20 mg ml^{-1} initial stock) pre-treatment (Estoup *et al*., 1996). "No-DNA" extractions were also conducted in order to check for contamination.

PCR (PTC-100® Peltier thermal cycler, MJ Research) of the second intron of the p26 gene was performed using the primers 262af (5′-ACGGAGGATT TGGTGGTATG-3′) and 263ar (5′-GGATAGTTA AGACGCCATCT-3′). These primers were designed based on the conserved regions of the second and third exons of p26 gene sequences of *A. franciscana* (available from GenBank, accession numbers DQ310 577, DQ310575, and AF031367). The PCR profiles and conditions were as follows: 4 min at 94 °C, 31 cycles of 50 sec at 94 °C, 50 sec at 66 °C, 1 min and 10 sec at 72 °C and a final extension of 7 min at 72 °C. Total reaction volumes of 25 µl consisted of 2.5 µl template DNA, 5 µl 5 × PCR buffer, 2.5 mM $MgCl_2$, 1 mM dNTPs, 0.001 mM of each primer and 1.25 U of *Taq* DNA polymerase. PCR products were extracted from agarose gels (2%), purified using the NucleoSpin Extract kit (Macherey-Nagel) and sequenced (direct sequencing and primer walking) on a PRISM 3730xl DNA analyzer (Applied Biosystems).

BLAST searches confirmed the identity of the PCR products on the basis of detected similarities with second and third exons of p26 sequences from other *Artemia* representatives as well as with the whole p26 gene of *A. franciscana* (DQ310575). For alignment, ClustalX (Thompson *et al*., 1997) was used with the following parameters: gap opening penalty = 90, gap extension penalty = 2, delay divergent sequence percentage = 60. In order to examine if the obtained sequences bear phylogenetic information, a substitution saturation test was employed (Xia *et al*., 2003).

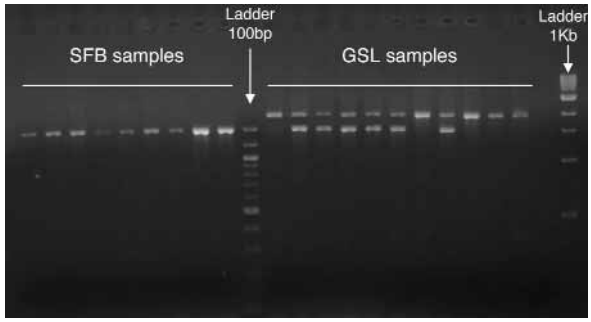

FIG. 1. PCR-amplified intron 2 fragments stained with EtBr and visualized under UV light. SFB samples: individuals from San Francisco Bay; GSL samples: individuals from Great Salt Lake.

RESULTS

Genetic analysis

Agarose gel electrophoresis revealed that the size of the PCR-amplified intron 2 fragment is different in the two populations. In particular, SFB *A. franciscana* yielded one fragment of ~1500 bp (Fig. 1). On the contrary, for GSL *A. franciscana*, two patterns were observed (visualized in agarose gels stained with EtBr under UV light); the first one produced a single band (~2000 bp, GSL_A), while the second pattern presented the GSL_A band along with a ~1500 bp band (GSL_B) (Fig. 1). This characteristic length variation was also further verified through primer walking and sequencing of three individuals (one from each pattern).

The SFB sequence (accession number FJ869882) was 1358 bp long (nucleotide composition: A = 30.2%, C = 17.5%, G = 19.0%, T = 33.3%). The GSL_A sequence (accession number FJ869884) was 1770 bp long (nucleotide composition: A=29.9%, C=17.3%, G = 20.2%, T = 32.5%) while the GSL_B sequence (accession number FJ869883) was 1355 bp long (nucleotide composition: A = 30.2%, C = 17.7%, G = 18.8%, T = 33.3%). No significant difference in nucleotide composition was detected ($\chi^2 = 1.25$, $p > 0.05$). A substitution saturation test for these three sequences (Xia *et al*., 2003) showed that the index of substitution saturation ($I_{SS} = 0.42$) was significantly lower ($T = 25.40$, $df = 1815$, $p < 0.05$) than the critical value ($I_{SS.C} = 0.83$). The identity of the amplified fragments was confirmed by BLAST searches [based on the similarities detected with one deposited sequence of intron 2 of p26 gene from *A. franciscana* (DQ310575) and homologies identified with the 3′ end of exon 2 and with the 5′ end of exon 3 from *A. franciscana* (AF031367, DQ310577), *A. persimilis* (DQ310578), *A. sinica* (DQ310576), and *A. urmiana* (DQ310580)].

The alignment length was 1816 bp. The alignment showed that there are two conserved regions at the beginning (Block A) and at the end (Block C) of the alignment (Fig. 2). More specifically, as BLAST showed, Block A consists of 148 bp; the first 28 bp belong to exon 2 of the p26 gene and the rest 120 bp belong to the intron 2 sequence. Similarly, Block C consists of 124 bp; the first 59 bp belong to the intron 2 sequence and the last 65 bp correspond to the 5′ end of exon 3. Furthermore, in the produced alignment, there is an insertion (403 bp long) in the GSL_A sequence (Block B) and eight indels that range from 3 to 35 bp (Fig. 2); these are responsible for the differences in the sizes of the PCR products. The comparison of the three sequences revealed that there were 25 transitional and 20 transversional pairs.

In total, all the SFB individuals (n = 50) presented the same size of the PCR-amplified product (~1500 bp). For GSL individuals, 23 individuals showed the GSL_A band, while the rest (n = 28) produced both bands (GSL_A/GSL_B pattern).

Breeding experiments

Out of 20 cross-breeding tests between the two populations, five were not successful. The remaining produced 34 to 270 nauplii, most of which (~65%) reached adulthood (Table 1). Also, the production of cysts was recorded in 4 crosses (Table 1). All parental and 30 individuals of each cross belonging to F_1 were scored genetically. All SFB parents showed one fragment of ~1500 bp whereas GSL parents produced either one band (GSL_A) or two bands (GSL_A/GSL_B). In the first case, when the GSL parent had only the GSL_A band (crosses 5, 6, 8, 9, 13, 14 and 15, see Table 1), all F_1 individuals showed two bands (each one was inherited from their parents, see Table

FIG. 2. Alignment of intron 2 of p26 gene. SFB: San Francisco Bay, GenBank Accession No FJ869882; GSL_B: Great Salt Lake, GenBank Accession No FJ869883; GSL_A: Great Salt Lake, GenBank Accession No FJ869884. Block A, Block B, and Block C denote conserved regions of the sequenced fragment (for more details, see text).

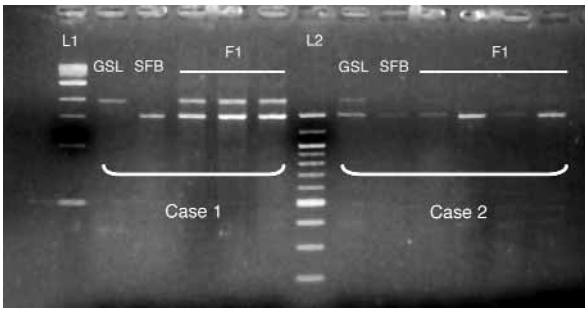

FIG. 3. PCR-amplified intron 2 fragments of GSL and SFB individuals (and their offspring). Case 1: GSL parent with GSL_A band and three F_1 individuals with two bands (for more details, see Table 1). Case 2: GSL parent with GSL_A/GSL_B pattern and four F_1 individuals with PCR patterns similar to the one or to the other parent (for more details, see Table 1). L1: ladder 1kb, L2: ladder 100bp.

1 and Fig. 3). In the second case, when the GSL parent showed the GSL_A/GSL_B pattern (crosses 2, 4, 7, 10, 11, 12, 16 and 17, see Table 1), F_1 individuals presented PCR patterns similar to the one or to the other parent (Table 1 and Fig. 3). The ratio was close to 1:1 (χ^2 test, see Table 1).

DISCUSSION

In the present study, we have tried to assess the quality and the potential application of a new genetic marker for identifying two *A. franciscana* populations, namely GSL and SFB. *Artemia franciscana* cysts originating from GSL cover ~90% of the global cyst demand for aquaculture purposes. On the other hand, *A. franciscana* from SFB has been used as inoculum

Table 1. Results of 20 reciprocal cross-breeding tests (GSL × SFB). Bold lettering indicates that the GSL parent showed the pattern GSL_A/GSL_B (for more details, see text). χ^2 test was used in order to check if the observed frequencies were significant different from the expected (by the Mendelian mode of inheritance) ones

Cross	No of broods	Total offspring		Produced patterns (nauplii)			
		cysts	nauplii	A[1]	B[1]	A/B[1]	$\chi^2_{(df=1)}$
GSL♀ × SFB♂ (1)	0	–	–	–	–	–	
GSL♀ × SFB♂ (2)	1	–	50	14	–	16	0.133*
GSL♀ × SFB♂ (3)	0	–	–	–	–	–	
GSL♀ × SFB♂ (4)	2	–	143	13	–	17	0.533*
GSL♀ × SFB♂ (5)	1	–	77	–	–	30	
GSL♀ × SFB♂ (6)	5	–	270	–	–	30	
GSL♀ × SFB♂ (7)	2	+	69	16	–	14	0.133*
GSL♀ × SFB♂ (8)	3	+	152	–	–	30	
GSL♀ × SFB♂ (9)	2	–	109	–	–	30	
GSL♀ × SFB♂ (10)	4	–	84	15	–	15	0*
SFB♀ × **GSL**♂ (11)	1	–	60	16	–	14	0.133*
SFB♀ × **GSL**♂ (12)	1	–	58	16	–	14	0.133*
SFB♀ × GSL♂ (13)	1	–	79	–	–	30	
SFB♀ × GSL♂ (14)	1	–	43	–	–	30	
SFB♀ × GSL♂ (15)	2	+	110	–	–	30	
SFB♀ × **GSL**♂ (16)	1	+	34	13	–	17	0.533*
SFB♀ × **GSL**♂ (17)	3	–	94	14	–	16	0.133*
SFB♀ × GSL♂ (18)	0	–	–	–	–	–	
SFB♀ × GSL♂ (19)	0	–	–	–	–	–	
SFB♀ × GSL♂ (20)	0	–	–	–	–	–	

* $p > 0.05$

[1] Pattern A: one PCR-amplified band at ~1500 bp; Pattern B: one PCR-amplified band at ~2000 bp, Pattern A/B: two PCR-amplified bands, one at ~1500 and one at ~2000 bp

in a number of projects aiming in cyst production (e.g. Brazil and Vietnam) and as a reference material for laboratory studies. Our results showed that these two populations can be discriminated according to the length difference of the PCR-amplified intron 2 of p26. More specifically, all SFB individuals (scored in this study) showed the same pattern (~ 1500 bp); almost 45% of GSL individuals produced a single band of ~ 2000 bp (GSL_A), while the rest showed the pattern GSL_A/GSL_B (two bands of ~ 1500 bp and ~ 2000 bp, respectively). It should be noted that the GSL_A band was never observed in any of the SFB individuals scored. The length difference between SFB and GSL_B and GSL_A sequences is attributed –to a large extent– to an insertion of ~ 400 bp (Block B, Fig. 2).

Large deletions and insertions (indels) cause shifts in the sizes of certain DNA regions. This phenomenon has been already observed in *Artemia* for the ITS1 region; length variation of PCR products is clade-specific and can be used for discriminating *A. persimilis* and *A. salina* from the remaining bisexual species and parthenogenetic strains (Baxevanis *et al*., 2006). The length variation of intron 2 of the p26 gene presented in this study may serve as a rough-and-ready marker for the identification of the two most commercially important populations (i.e. SFB and GSL). It should be noted that the length of the amplified ITS1 region for both populations is the same (Baxevanis *et al*., 2006). Furthermore, since indels are unlikely to arise from mutational mechanisms applied to substitutional data (Pons & Vogler, 2006), smaller gaps (1-30 bp) are hypothesized to result from slipped-strand mispairing while larger gaps (>30 bp) are caused by unequal crossing-over or are due to transposition (Giribet & Wheeler, 1999; Creer, 2007). Therefore, the indel of ~ 400 bp observed between SFB and GSL may represent a conspicuous differentiation event.

Although preliminary, the results produced by the substitution saturation test ($I_{SS} < I_{SS.C}$) revealed that the analysed sequences have not experienced severe substitution saturation. Therefore, the genetic marker proposed in this study has not lost its phylogenetic signal due to substitution saturation (Xia *et al*., 2003). It should be noted that the results of the substitution saturation test may be biased, since only intraspecific data were used. In order to fully explore its potential use for phylogenetic studies, further analyses are needed (with the inclusion of more *A. franciscana* populations and/or *Artemia* species).

Liu & Cordes (2004) reported that the genetic variation (in order to be useful from a geneticist point of view) should be 1) heritable and 2) discernable to the researcher through molecular techniques. The mode of inheritance of intron 2 is well established since the offspring produced by reciprocal crosses (SFB × GSL) follow the Mendelian mode of inheritance; the expected ratios do not differ significantly from the observed ones. For the successful reciprocal crosses between these two *A. franciscana* populations, the observed fecundity (51.5 ± 16.80 nauplii per brood, see Table 1) was far below that recorded for intra-population cross-fertility tests of the same species (~ 300 nauplii per brood, Ruebhart *et al*., 2008). This fact may indicate the initial stage of genetic barriers between SFB and GSL populations supporting the occurrence of incipient speciation in *A. franciscana* (Triantaphyllidis *et al*., 1998 and references therein).

Furthermore, the limited survival of F_2 and F_3 nauplii (< 10%, data not shown) indicate a different type of reproductive barrier, namely hybrid breakdown: inviability or sterility observed only in the F_2 or later generations of interspecific or intersubspecific crosses, while F_1 hybrids are viable and fully fertile –at least under laboratory conditions– (Abatzopoulos *et al*., 1998, 2002; Oka *et al*., 2004). This phenomenon may be attributed to the disruption of interaction of genes at different loci as genes segregate after the F_1 generation. According to Oka *et al*. (2004) "Hybrid breakdown is (thus) hypothesized to arise when genetic segregation causes alleles of each interacting locus to become homozygous in an improper way". Consequently, the hybrid breakdown may be considered as a recessive trait (Orr, 1993). According to our results, none of the GSL individuals from the wild population showed exclusively the GSL_B band (all scored individuals presented either GSL_A or GSL_A/GSL_B patterns). Given i) the limited differences between SFB and GSL_B sequences (Fig. 2) and ii) the viable and fertile F_1 hybrids produced by SFB × GSL reciprocal crosses (Table 1) and the low survival of F_2 and F_3 nauplii, the absence of single GSL_B pattern from the wild GSL population could be attributed to a potential hybrid breakdown mechanism existing in F_2 and/or later generations.

Another intriguing observation is the fact that the GSL_A pattern was never observed in the SFB wild population. A number of reasons may elucidate the "one-way invasion" from SFB to GSL; i) the SFB population is more eurythermal than the GSL population (SFB population has been successfully used in

inoculation projects around the globe, see Kappas *et al*., 2004 and Camara *et al*., 2005) and ii) the GSL cysts require more complex pathways for diapause deactivation compared with the SFB cysts (Lavens & Sorgeloos, 1987). The greater phenotypic plasticity (regarding temperature) of *A. franciscana* SFB population (compared with that of GSL population) could be expressed by its significant capability to expand in niches with high diversity.

In conclusion, the marker described in this work is a useful tool (in terms of high speed, reduced cost and low complexity) in discriminating the *Artemia* populations from SFB and GSL, although further studies are needed (more populations to be scored) in order to expand its application within the species and/or the genus. The proposed genetic marker may be effectively applied in aquaculture industry (genetic characterization of the commercial strains from USA and/or the genetic follow-up of inoculation efforts), in molecular ecology (dispersal of cysts via waterfowl and/or invasion of *A. franciscana* strains from North America) and in other aspects of applied research (as a crude marker for rapid and relatively reliable genetic characterization).

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