Experimental hybridization of two African *Streptocephalus* species (Crustacea, Branchiopoda: Anostraca)

Henri J. Dumont* and Els Adriaens

Laboratory of Animal Ecology, University of Gent, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium

Fairy shrimp are ancient crustaceans that live in islands of ephemeral waters all over the world. Although an ancient group, there is evidence that they evolve slowly and thus, genetic differences between species can be relatively small.

Here, we hybridize two African species of *Streptocephalus* with overlapping geographical ranges, and find that hybrids have reduced viability. We postulate a model of allopatric speciation, and discuss what may happen if sympatry is secondarily regained. We argue that strong pre- and post-mating barriers should be favoured if speciation has proceeded in conditions where the risk of sympatry is non-negligible. In species that evolved in absolute allopatry (like in Africa and North America), no selection for such barriers exists, however. Man nowadays increasingly interferes with incipient speciation by artificially connecting ephemeral pools, in which case full introgression may occur.

Keywords: Broods, cysts, hybridization, laboratory cultures, *Streptocephalus* species.

THE large branchiopod genus *Streptocephalus* inhabits temporary waters of Africa, Eurasia, Australia, and North and Central America. It is an ancient group, whose origins can be traced to Gondwanaland[1]. The American and African species have lived in isolation for millions of years. Yet hybrids, not only between American species, but also between at least one American (*S. mackini*) and one African species (*S. bouvieri*) under laboratory conditions have been obtained relatively easily[2,3], with some hybrids being fertile. This, at first sight, suggests a slow rate of evolution. In some broadly sympatric North-American species-pairs, hybridization has been reported with fertile hybrids, while in some cases hybridization was futile. This suggests a full gradation of the speciation process to be present in the American species. Wiman[2,3] also hypothesized that in African *Streptocephalus*, a similar plethora of hybridizations might be expected. In the present study, two species of *Streptocephalus*, *S. torvicornis* and *S. rubricaudatus* were used. The first is widely distributed in northern Africa, around the Mediterranean, and in West Asia. The second is typical of the African Sahel, and their ranges meet and overlap in the central Sahara. The experiments in the present study were conducted using cultures derived from dried cysts collected in Central Algeria, Tassili-n-Ajjer area, where the two live in sympatry in the lakelet (guelta) of Azar, Dider.

Laboratory cultures derived from cysts collected from the wild (Guelta Azar) were set up, and specimens of both sexes were isolated as larvae and grown to sexual maturity in isolation. In mating experiments, ten replicates each of reciprocal tests were set up, in which either one female of *S. rubricaudatus* in a beaker with 400 ml of water was exposed to two males of *S. torvicornis*, or vice versa. The experiment lasted for 15 days. Two males were allotted to one female to introduce an element of male competition. Broods produced were immediately removed, dried and counted. After a few weeks they were re-incubated and any hybrids that hatched were raised to adulthood under similar conditions as pure lines (temperature 25°C, baker's yeast, *Chlorella* and *Scenedesmus*).

When *S. rubricaudatus* females were mated with *S. torvicornis* males, 22 broods were obtained, out of which the total broods with viable cysts was 15. The percentage of females producing cysts was 90. In contrast, the reciprocal matings yielded 21 broods, out of which broods with viable cysts were 8 and the percentage of females producing cysts was 70.

All matings produced hybrids, similar in terms of the number of broods obtained, but the percentage of broods with viable cysts and the number of females producing cysts were higher with *S. rubricaudatus* females mated to *S. torvicornis* males than in the reverse case. A *t*-test carried out on the means revealed significant differences ($P < 0.001$). The average brood size was higher in *S. rubricaudatus* females (81), as well as the absolute maximum number of cysts per clutch (283), and both were considerably lower than the average maximum brood sizes of pure cultures of *S. torvicornis*, where clutch sizes of 4–500 cysts are no exception (pers. obs.). The average brood size was 61 with a maximum brood size of 136, when *S. torvicornis* were used as females. The hatching percentage of hybrid cysts was higher when *S. rubricaudatus* was used as females (16) compared to 6 when *S. torvicornis* was used as females. Of the hatched nauplii,

*For correspondence. (e-mail: Henri.Dumont@ugent.be)

50% died before reaching adult size, and adults were short-lived. Any attempts to cross the hybrids failed. A morphological investigation showed that the clasping antennae of the males lacked denticulation and showed numerous monstrosities. Also, the endites of the filtering trunk limbs had a strongly reduced setulation. Since these microsetules have a species-specific shape[4], they determine which type of particles will be retained, and are vital to the filtering performance of the limbs. Under these circumstances the animals may in fact have died of starvation, while swimming amidst food[5].

Intra-generic hybridization is not uncommon in Anostraca, and has been recorded in *Branchinecta*[6] and *Artemia*[7,8]. Under laboratory conditions, when *B. belki* was mated with *B. packardi*, a fertile F_1 was obtained, which on inter se mating produced cysts. The progeny thus obtained from inter-stock matings were morphologically intermediate between the two parents[6]. The F_2 was not further tested, but ovigerous females were obtained. In *Artemia*, where gross morphology is similar across the genus, hybrids were merely distinct in reverting from being predominantly ovoviviparous in the parents to being oviparous[8]. In *Streptocephalus*, the situation is either more complex or has been better studied. Although the Streptocephalidae are a complex and speciose group, they are considered to include only one genus, *Streptocephalus*, divided into several evolutionary lineages[9,10]. Some of these lineages, especially those that occur on different continents, may deserve generic rank. Thus, if genetic distance is a measure of genetic dissimilarity, one might expect hybridization to be more difficult between than within lineages. However, this is not systematically true, and in fact, the reverse sometimes occurs. *S. rubricaudatus* and *S. torvicornis* are related and belong to the same lineage, yet their hybrids were difficult to obtain and had limited viability. Pre- as well as post-mating barriers to hybridization seem to be strong here, and ensure that wasteful hybridization in these sympatric congeners either does not occur or is reduced to a minimum. Of the six species used by Wiman[2,3], including five from North America and one (*S. bouvieri*) from Africa, few produced no hybrids at all, confirming three species, *S. dorothae*, *S. taxanus* and *S. seali* as reproductively fully isolated from each other. Like in *S. torvicornis* and *S. rubricaudatus*, the ranges of these three species overlap, and their probability of co-occurrence is high. All of them more or less freely mated with *S. mackini*, a southern species that penetrates the ranges of the other species in the south. Remarkably, *S. mackini* also mated with the African *S. bouvieri*, with *S. mackini* males fertilizing more *S. bouvieri* females than did *S. bouvieri* males, and F_1 hybrids, whose fertility was not tested.

Limited data on the molecular genetics of Anostraca (the 18S rDNA gene sequence) reveal the gene as remarkably conserved across the order (gene length 1806–1810 bp)[11], in stark contrast with the other branchipod orders, where as well gene length as sequence order varies strongly. Seven species of *Streptocephalus* analysed phylogenetically included *S. torvicornis* and *S. dorothae*. Both branched out as extremes of the family tree, but sequence variation within the Streptocephalidae as a whole was extermely low (0.001–0.006 substitutions per site, compared to up to 0.020 substitutions per site in other anostracans). If this gene is representative of the entire genome, it follows that the Streptocephalidae are indeed subject to slow evolution, and species demarcations are subject to the presence of mating barriers rather than genetic incompatibility of their genomes per se. Such mating barriers are most likely to emerge where strong selection against hybridization is present. One would expect such a situation to arise, where speciation has proceeded in allopatry, and two sister species secondarily regain sympatry[2,3,6]. Indeed, without such barriers, introgression would occur, as probably has happened and continues to happen on numerous occasions. But if two incipient taxa are separated from the start by a hermetic geographic barrier like an ocean, secondary sympatry will not occur, and the need for erecting barriers to mating will not arise. This partly explains the observations discussed here: the ease of hybridization of *S. mackini* with *S. bouvieri* may reflect the long isolation of both taxa, not subject to any threat of wasting energy to hybridization, while the laborious hybridization between *S. torvicornis* and *S. rubricaudatus* can be seen as the result of the opposite selective force, viz. two related sympatric species which are currently shielded from hybridization by an effective reproductive isolation. The case of the freely hybridizing *Branchinecta* of Mexico and the southern US, finally, is suggestive of an incipient speciation, still proceeding in allopatry, and in which secondary sympatry may currently lead to introgression.

1. Daniels, S. R., Hamer, M. and Rogers, C., Molecular evidence suggests an ancient radiation for the fairy shrimp genus *Streptocephalus* (Branchiopoda, Anostraca). *Biol. J. Linn. Soc.*, 2004, **82**, 313–327.
2. Wiman, F. H., Hybridisation and the detection of hybrids in the fairy shrimp genus *Streptocephalus*. *Am. Midl. Nat.*, 1979, **102**, 149–156.
3. Wiman, F. H., Mating patterns and speciation in the fairy shrimp genus *Streptocephalus*. *Evolution*, 1979, **33**, 172–181.
4. Beladjal, L., Mertens, J. and Dumont, H. J., An analysis of the setation pattern of the limbs in Anostraca (Crustacea), using the Algerian species as an example. *Hydrobiologia*, 1995, **298**, 183–201.
5. Beladjal, L. and Martens, J., Interstitial remains for fauna reconstruction of desert pools using fairy shrimps as example (Anostraca). *J. Crust. Biol.*, 2003, **23**, 60–68.
6. Maeda-Martinez, A., Obregon-Barboza, H. and Dumont, H. J., *Branchinecta belki* n sp. (Branchiopoda: Anostraca), a new fairy shrimp from Mexico, hybridizing with *B. packardi* Pearse under laboratory conditions. *Hydrobiologia*, 1992, **239**, 151–162.
7. Bowen, S. T., Fogarino, E. A., Hitchner, K. N., Dana, G. L., Chow, V. H. S., Bouncristiani, M. R. and Carl, J. R., Ecological isolation in *Artemia*: population differences in tolerance of anion concentrations. *J. Crust. Biol.*, 1985, **5**, 106–129.

8. Gajardo, G., Parraguez, M., Beardmore, J. A. and Sorgeloos, P., Reproduction in the brine shrimp *Artemia*: evolutionary relevance of laboratory cross-fertility tests. *J. Zool.*, 2001, **253**, 25–32.
9. Daday, E. De, Monographie systematique des phyllopodes anostaces. *Ann. Sci. Nat., Zool.*, 1910, **11**, 91–489.
10. Maeda-Martinez, A. M., Belk, D., Obregon-Barboza, H. and Dumont, H. J., A contribution to the systematics of the Streptocephalidae (Branchiopoda: Anostraca). *Hydrobiologia*, 1995, **298**, 203–232.
11. Weekers, P. H. H., Murugan, G., Vanfleteren, J. R., Belk, D. and Dumont, H. J., Phylogenetic analysis of anostracans (Branchiopoda: Anostraca) inferred from nuclear 18S ribosomal DNA (18S rDNA) sequences. *Mol. Phylogenet. Evol.*, 2002, **25**, 535–534.

MEETINGS/SYMPOSIA/SEMINARS

National Conference on Global Climatic Change and its Impact on Biodiversity

Date: 19–20 February 2009
Place: Palayamkottai

Topics include: Biodiversity – flora and fauna; Land degradation and GIS; Water management; Soil management; Forest management; Biotechnology and bioinformatics; Geo-technology and Geo-informatics; Social economic status; Vulnerability and poverty; Ecosystem; Consumer choice, Industrial policies and Environmental sustainability; Research and innovation for energy and the environment.

Contact: Dr L. Louis Jesudass
Organizing Secretary
National Conference on Climatic Change and its Impact on Biodiversity
St Xavier's College (Autonomous)
Palaymkottai 627 002
Ph (O): 0462-4264316
Mobile: 094434 52430
Fax: 0462-2561765
E-mail: sxcconference09@yahoo.com

Workshop on the Digitalized CD ROM Animal Alternatives to Replace the Animals used in Zoology Practical Curriculum

Date: 5 February 2009
Place: Sivakasi

Training and hands on experience with animal alternative CDs (frog, earthworm, shark, rat, pigeon, crayfish) using computers.

Eligibility: Lecturer/Reader in Zoology of Colleges/Universities

No. of participants: 50 (first come first served basis); No registration fee.

Contact: Dr S. Baskaran
PG and Research Department of Zoology
Ayya Nadar Janaki Ammal College (Autonomous)
Sivakasi 626 124
Ph: 04562 254100
Fax: 04562 254970
Mobile: 98432 99550
E-mail: vgr_anjac@sancharnet.in
Website: www.anjac.org