Hybridization generates a hopeful monster: a hermaphrodic selfing cichlid

Ola Svensson¹,², Alan Smith¹, Javier García-Alonso¹,³ and Cock van Oosterhout⁴

¹School of Biological, Biomedical and Environmental Sciences, University of Hull, Hull, UK
²Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, Gothenburg 405 30, Sweden
³Biodiversity Group, Centro Universitario Regional Este, Universidad de la República, Maldonado 20000, Uruguay
⁴School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, UK

Compared with other phylogenetic groups, self-fertilization (selfing) is exceedingly rare in vertebrates and is known to occur only in one small clade of fishes. Here we report observing one F1-hybrid individual that developed into a functional hermaphroditic after crossing two closely-related sexually reproducing species of cichlids. Microsatellite alleles segregated consistent with selfing and Mendelian inheritance and we could rule out different modes of parthenogenesis including automixis. We discuss why selfing is not more commonly observed in vertebrates in nature, and the role of hybridization in the evolution of novel traits.

1. Introduction

Functional hermaphroditism is common in both plants and animals but is exceedingly rare among vertebrates [1]. The vertebrate exception is teleost fishes in which functional hermaphroditism has been reported in 27 families in seven orders, predominantly among tropical marine perciforms [2]. Self-fertilization (selfing) is much rarer than hermaphroditism, although this too is found in many animal and plant taxa [3]. In vertebrates, there are more than 80 known taxa of vertebrates that reproduce without sex [4], whereas selfing has only been observed in the clade with two species of Kryptolebias [2,5]. In teleost fish, all known parthenogenic lineages have hybrid origin [6].
Figure 1. (a) The intersex produced by a cross between a female *Pundamilia pundamilia* from Python Island and an orange blotch (OB) *Neochromis omnicaeruleus* male from Makobe Island. The gonads (b) looked like typical cichlid ovaries. However, a horizontal plane section (c) contained small aggregated dark dots that resembled spermatocytes (marked by arrow). On the gonad (b), this section was located in a whitish tissue (arrow).

In cichlids, the heterogametic sex differs between species, and the sexes are environmentally determined in some species (e.g. by pH or temperature), which can occur even in the presence of genetic sex determination [7]. Sex-determination systems turnover in conjunction with female orange blotch (OB) polymorphism are thought to have contributed to the formation of haplochromine cichlid species flocks in the African great lakes [8,9]. Sex change has been suggested in *Crenicara punctulata* [10], and in *Cichlasoma portalegrense* there is a description of a fully developed intersex gonad [11]. Here we report an individual cichlid that was produced in an F1-hybrid cross in a study on the OB polymorphism and sex determination. Uniquely, this individual reproduced fertile female and male offspring by hermaphroditic selfing.

2. Material and methods

Females of *Pundamilia* (*Haplochromis*) *pundamilia* and *Pundamilia* (*Haplochromis*) *nyererei* were crossed with OB-coloured males of *Neochromis* (*Haplochromis*) *omnicaeruleus* (*N* = 31 crosses). These closely-related, often sympatric, species have an estimated divergence time of 0.1–0.35 million years [12]. When 81 F1 female hybrids were kept in isolation for one to six weeks (to be photographed), one female (‘intersex’, *P. pundamilia* × *N. omnicaeruleus*, figure 1a) spawned. In total, this intersex produced 14 broods, raising 46 living F2-juveniles over a period of 25 months while in isolation in a 20 × 20 × 20 aquarium. Two sons and 15 daughters survived to adulthood and were fertile. The intersex was then euthanized using an overdose of MS222 for morphological and histological analyses. As an effort to find further selfing individuals, all 18 full-sib sisters and 12 daughters of the intersex were kept in isolation for 12 months.

The intersex, its parents and the first four offspring were genotyped at nine microsatellite loci (Ppun5, Ppun7, Ppun17, Ppun21, Ppun32, Pzeb1, UME003, UNH002 and UNH130). Methods for DNA extraction and PCR reactions were as described previously [13,14]. Samples were genotyped on a Beckman Coulter CEQ 8000 capillary sequencer and genotypes were determined manually. We wrote a computer model to investigate whether the observed heterozygosity (Ho) of the offspring was consistent with selfing, or whether inbreeding with a (simulated) full sibling could also explain Ho. The model calculates Ho across nine microsatellites of four simulated offspring and assumes that the intersex produced with a simulated full sib (generated by the model based on the parents’ genotypes). This procedure was repeated 10 000 times. The Ho of the four genotyped offspring was then compared with the distribution of simulated Ho values.

The gonads were fixed for 24 h in Bouin’s solution or 4% paraformaldehyde solution, embedded in paraffin and serially sectioned (horizontal plane) at 5 and 7 µm. Sections were stained with haematoxylin and eosin to identify oogenic and spermatogenic tissue. Slides were examined with an Axiolab (Zeiss) light microscope and photographed.
3. Results

All alleles of each offspring were present in the intersex (table 1) and together with the fact that the intersex was held in isolation, we can for each analysed offspring confidently exclude all known modes of reproduction in vertebrates except selfing. Other forms of reproduction without a mate would result in either genetically identical clones, or individuals with a near-complete homozygous genome (automixis with terminal meiotic fusion) or heterozygous genome (automixis with central fusion) [6,15–19]. The intersex was heterozygous for the nine microsatellite loci and the mean observed heterozygosity (Ho) of the four offspring at these loci was Ho = 0.444 (table 1). This value is approximately equivalent to the level of heterozygosity expected from selfing of a completely homozygous individual (Ho = 0.5). We modelled the distribution of Ho values based on the simulated full-sib mating in a computer model which showed that the observed value is significantly lower than the simulated heterozygosity (Ho (5–95% CI) = 0.746 (0.611–0.861); p < 0.001). Thus, the observed level of inbreeding was significantly higher than inbreeding resulting from non-selfing sexual reproduction, even if mating had occurred with the most closely-related individual possible (i.e. a full sibling).

The ovaries of the intersex female were located dorsally to the gut and filled a large volume of the peritoneal cavity. In the histological samples, ovarian tissue contained oogonia and oocytes in all stages of germ cell development. However, a whitish tissue in the antero-ventral part of the gonad (figure 1b) without any apparent barrier to the ovarian tissue showed an unusual aggregation of small dark dots. These dots had an appearance similar to spermatocytes (figure 1c).

3.1. Adult offspring

Of the 46 F2-offspring of the intersex female, three developed male nuptial coloration, and 15 showed female nuptial coloration after 12 months, which deviates significantly from an equal sex ratio (binomial test, p = 0.0075). Twenty-eight individuals could not be sexed because they died when juveniles (58.5% mortality), which was higher than the (mean ± s.d.) 7.6 ± 13.1% mortality in F1-crosses between the same two species as the intersex female (RB Stelkens, personal communication, [20]). When crossed, both surviving F2-males successfully spawned with 10 of the 15 sisters and produced offspring of both sexes with an approximately equal sex ratio (15 males and 18 females, binomial test, p = 0.73). This F3-generation also showed reduced survival (45% juvenile mortality), but proved to be fertile. None of the 18 full-sib sisters of the intersex, nor any of the 12 daughters, reproduced whilst kept in isolation.

4. Discussion

An individually housed F1-hybrid cichlid (intersex) produced viable and fertile offspring of both sexes without access to sperm from another individual. Microsatellite DNA analyses showed no evidence for aberrant chromosome numbers, i.e. the intersex and its offspring were diploid, and their observed heterozygosity (Ho) was consistent with Mendelian segregation after self-fertilization (selfing). Furthermore, the Ho was significantly lower than a hypothetical single generation of full-sib mating (i.e. the observed level of inbreeding was more severe). Sexually reproducing vertebrates occasionally produce offspring through facultative parthenogenesis [18,19], which appears to be realized through automixis where the terminal meiotic products fuse causing genome wide homozygosity [18,19]. Central fusion automixis is not known in vertebrates and typically all offspring are females with extensive

| Table 1. Microsatellite DNA analysis. |
|--------------------------------------|
| Individual | Pmin5 | Pmin7 | Pmin17 | Pmin21 | Pmin32 | Pzeb1 | UME003 | Unh002 | UNH130 |
|-----------|-------|-------|--------|--------|--------|-------|--------|--------|--------|
| Mother    | 204/220 | 217/261 | 105/113 | 356/377 | 155 | 155/183 | 245/262 | 228 | 215/219 |
| Father    | 196/236 | 241/269 | 117/133 | 317/365 | 157/161 | 231 | 241/249 | 220 | 181/188 |
| Intersex  | 220/236 | 217/269 | 105/133 | 317/377 | 155/157 | 183/231 | 245/249 | 220/228 | 188/219 |
| Offspring 1| 236 | 217 | 105 | 377 | 155/157 | 183/231 | 245/249 | 220/228 | 188 |
| Offspring 2| 236 | 269 | 105/133 | 377 | 155/157 | 183/231 | 249 | 220 | 219 |
| Offspring 3| 236 | 217/269 | 133 | 317/377 | 155 | 183/231 | 249 | 220/228 | 219 |
| Offspring 4| 220/236 | 217/269 | 133 | 317 | 155/157 | 231 | 245/249 | 220/228 | 219 |
female-biased sex ratio in those F2-hybrids. Such bias in sex ratio is consistent with Haldane's rule [33] from inbreeding depression resulting in high juvenile mortality. Furthermore, there was a significant consequences of selfing. In the present study, the offspring of the intersex female appeared to suffer of predominant selfing with occasional outcrossing [32], which may partially offset the deleterious selfing vertebrates (selfing lineage. Indeed, as in many selfing plants [31], rather than obligate selfing, the only known selfing in mammals and birds. In teleosts, however, hermaphroditism is relatively common and intersex does occasionally occur in nature [2,30], which suggests that selfing lineages do evolve. Yet, a selfing lineage is likely to be short-lived because recessive deleterious mutations will become expressed in homozygous state which increases the extinction risk [31]. Given that many vertebrate species have a low reproductive potential, selection will be inefficient in purging deleterious mutations from a selfing lineage. Indeed, as in many selfing plants [31], rather than obligate selfing, the only known selfing vertebrates (Kryptolebias marmoratus and K. hermaphroditus) have evolved a mixed mating strategy of predominant selfing with occasional outcrossing [32], which may partially offset the deleterious consequences of selfing. In the present study, the offspring of the intersex female appeared to suffer from inbreeding depression resulting in high juvenile mortality. Furthermore, there was a significant female-biased sex ratio in those F2-hybrids. Such bias in sex ratio is consistent with Haldane’s rule [33] which stipulates that the heterogametic sex tends to be absent or rare in hybrids. However, because the parents of the intersex female (non-OB × OB) were likely to possess different sex determining genes with different heterogametic sexes [8,9], and because the F3-hybrids showed no deviation in sex ratio, we do not want to speculate whether or not the observed female bias in the F2-hybrids is due to Haldane’s rule, aggression based sex biased mortality, or deviation due to conflicting sex-determination systems.

To conclude, one F1-hybrid individual developed into a functional selfing hermaphrodite after crossing two closely-related sexually reproducing species of cichlids. Hermaphroditic selfing is likely to be underreported in vertebrates because of the unusual set of conditions that is required for it to be observed. Our study shows that facultative selfing is possible in teleost fishes, and we suggest that although such ‘hopeful monsters’ may be evolutionarily transient and rare in nature, certain environmental conditions may make them biologically significant.

Ethics. The experiment described complies with the Guidelines of the European Union Council (86/609/EU) and the Bioethical Committee of the University of Hull (UK) for the use of laboratory animals. This work was performed under a UK government Home Office licence (PPL 60/3295).

Data accessibility. All raw data are included in the article. The model code is available from C.v.O. upon request.

Authors’ contributions. O.S. carried out the microsatellite analysis, A.S. the breeding and fish maintenance, J.G.-A., dissection and histology and C.v.O. statistical analysis and computer modelling. O.S. and C.v.O. analysed the data and drafted the manuscript with contributions from A.S. and J.G.-A. All authors gave final approval for publication.

Competing interests. We have no competing interests.

Funding. O.S. was supported by the Linnaeus Centre for Marine Evolutionary Biology at the University of Gothenburg (http://www.cemeb.science.gu.se/) and C.v.O. was supported by the Earth and Life Systems Alliance (ELSA).
References

1. Policansky D. 1982 Sex change in plants and animals. Annu. Rev. Ecol. Syst. 13, 471–495. (doi:10.1146/annurev.es.13.100182.002351)

2. Sadovy de Mitcheson Y, Liu M. 2008 Functional hermaphroditism in teels. Fish Fish. 9, 1–43. (doi:10.1111/j.1467-2979.2007.00266.x)

3. Jamre P, Auld JR. 2006 Animals mix it up too: the distribution of self-fertilization among hermaphroditic animals. Evolution 60, 1816–1824. (doi:10.1554/06-246.1)

4. Neaves WB, Baumann P. 2011 Unisexual reproduction among vertebrates. Trends Genet. 27, 81–88. (doi:10.1016/j.tig.2010.12.002)

5. Tatarenkov A, Earley RL, Taylor DS, Avise JC. 2012 Microevolutionary distribution of isogenicity in a self-fertilizing fish (Kryptolebias marmoratus) in the Florida Keys. Integr. Comp. Biol. 52, 743–752. (doi:10.1093/icb/icx075)

6. Lamatsch DK, Stöck M. 2009 Sperm-dependent reproduction among Lake Malawi cichlid fish populations is not simply predictable from male nuptial colour. BMC Evol. Biol. 9, 283. (doi:10.1186/1471-2148-9-283)

7. Oldfield RG. 2005 Genetic, abiotic and social influences on sex differentiation in cichlid fishes and the evolution of sequential hermaphroditism. Fish Fish. 6, 93–110. (doi:10.1111/j.1467-2979.2005.00184.x)

8. Parnell NF, Streelman JT. 2013 Genetic interactions controlling sex and color establish the potential for sexual conflict in Lake Malawi cichlid fishes. Heredity 110, 239–246. (doi:10.1038/hdy.2012.73)

9. Lane R, Seehausen O, van Alphen JIM. 2001 Mechanisms of rapid sympatric speciation by sex reversal and sexual selection in cichlid fish. Genetics 157, 435–443. (doi:10.1038/sj/jeb.7200138)

10. Carruth LL. 2000 Freshwater cichlid Crenicara punctulata is a protogynous sequential hermaphrodite. Copeia 2000, 71–82. (doi:10.1643/0045-8511(2000)0001(0071:FCPPA2.0.CO;2)

11. Polder JJW. 1971 On gonads and reproductive behaviour in the cichlid fish Aequidens portalegros (Rensch). Neth. J. Zool. 21, 265–365. (doi:10.1163/00282969708010503)

12. Stelkens RB, Schmid C, Selz O, Seehausen O. 2009 Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. BMC Evol. Biol. 9, 283. (doi:10.1186/1471-2148-9-283)

13. Blais J, Pienenderleith M, Rico C, Taylor MI, Seehausen O, van Oosterhout C, Turner GF. 2009 Assortative mating among Lake Malawi cichlid fish populations isaprotogynoussequential hermaphrodite. BMC Evol. Biol. 9, 53. (doi:S11186/1471-2148-9-53)

14. Stelkens RB, Pierotti MER, Joyce DA, Smith AM, van der Stuijs J, Seehausen O. 2008 Disruptive sexual selection on male nuptial coloration in an experimental hybrid population of cichlid fish. Phil. Trans. R. Soc. B 363, 2861–2870. (doi:10.1098/rstb.2008.0049)

15. Stalker HD. 1954 Parthenogenesis in Drosophila. Genetics 39, 4–34.

16. Stalker HD. 1956 On the evolution of parthenogenesis in Lonchopeta (Diptera). Evolution 10, 345–359. (doi:10.2307/2406996)

17. Verma S, Rutte F. 1983 Cytological analyses of the thelytokous parthenogenesis in the cape honeybee (Apis mellifera capensis). Apidologie 14, 41–57. (doi:10.1051/apido:19830104)

18. Booth W, Schuett GW, Ridgway A, Buxton DW, Castor TA, Bastone G, Bennett C, McMahnn W. 2014 New insights on facultative parthenogenesis in pythons. Biol. J. Linn. Soc. 112, 461–468. (doi:10.1111/bij.12286)

19. Lampert KP. 2008 Faculative parthenogenesis in vertebrates: reproductive error or chance? Sex. Dev. 2, 290–301. (doi:10.1007/s11955678)

20. Stelkens RB, Young KA, Seehausen O. 2010 The accumulation of reproductive incompatibilities in African cichlid fish. Evolution 64, 617–632. (doi:10.1111/j.1558-5646.2009.00849.x)

21. Oldfield RG. 2011 Genod development in Nidias cichlids and the evolution of sex change in fishes. Evol. Dev. 13, 352–360. (doi:10.1111/j.1525-142X.2011.00490.x)

22. Spurway H. 1957 Hermaphroditism with self-fertilization, and the monthly extrusion of unfertilized eggs, in the viviparous fish Lebiasit reticulatus. Nature 180, 1248–1251. (doi:10.1038/1801248a0)

23. Kawamura K, Hosoya K. 2000 Masculinization mechanism of hybrids in bumblerings (Telester: Cynipidae). J. Hered. 91, 464–473. (doi:10.1093/jhered/91.6.464)

24. Nichols P, Gennier MJ, van Oosterhout C, Smith A, Parsons P, Sungur H, Swanson J, Joyce DA. 2015 Secondary contact seeds phenotypic novelty in cichlid fishes. Proc. R. Soc. B 282, 20142272. (doi:10.1098/rspb.2014.2272)

25. Dittrich-Reed DR, Fitzpatrick BM. 2013 Transgressive hybrids as hopeful monsters. Evol. Biol. 40, 310–315. (doi:10.1007/s11759-010-0109-0)

26. McMullan M et al. 2015 Evidence for suppression of immunity as a driver for genomic introgressions and host range expansion in races of Albigna candida, a generalist parasite. eLife 4, e04550. (doi:10.7554/eLife.04550)

27. Seehausen O. 2004 Hybridization and adaptive radiation. Trends Ecol. Evol 19, 198–207. (doi:10.1016/j.tree.2004.01.003)

28. Devlin RH, Nagahama Y. 2002 Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208, 191–364. (doi:10.1016/s0044-8486(02)00057-7)

29. Bull JJ, Charnov EL. 1985 On irreversible evolution. Evolution 39, 1149–1155. (doi:10.2307/2408742)

30. Atz JW. 1964 Intersexuality in fishes. In Intersexuality in vertebrates including man (eds CN Armstrong, AJ Marshall), pp. 165–232, London, UK: Academic Press.

31. Wright SJ, Kalisz S, Slotte T. 2013 Evolutionary consequences of self-fertilization in plants. Proc. R. Soc. B 280, 20130133. (doi:10.1098/rspb.2013.0133)

32. Mackiewicz M, Tatarenkov A, Perry A, Martin JR, Elder JF, Bechler DL, Avise JC. 2006 Microsatellite documentation of male-mediated outcrossing between inbred laboratory strains of the self-fertilizing marine killifish (Kryptolebias marmoratus). J. Hered. 97, 508–513. (doi:10.1093/jhered/es1017)

33. Haldane J. 1922 Sex ratio and unisexual sterility in hybrid animals. J. Genet. 12, 101–109. (doi:10.1017/S0021851800084180)

Acknowledgements. We thank Ole Seehausen for support, Anssi Saura for advice on the discussion of automixis, and Joanna Wilson, Adelino VM. Canario and Africa Gomez for valuable comments on an earlier draft of the manuscript.