Courtship plasticity reveals the evolution of dialects in allopatric fish populations

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Traditionally, reproductive isolation between disjoint populations has been thought to emerge as a result of the accumulation of different mutations, genetic drift, or through the effects of natural and sexual selection\(^1\). Alternatively, the ability of an organism to express different phenotypes depending on the environment (i.e. phenotypic plasticity) could produce reproductive isolation\(^2,3\). Sexually selected traits are expected to be phenotypically plastic and can result in modifications of the species recognition system and thus originate new species\(^4\). Here we show that the population-characteristic male courtship behaviour of a fish (Girardinichthys multiradiatus) is modified in the presence of females from other populations, that this is due to the males responding to subtle cues from females, and that they fail to emulate the female's population-characteristic behaviour. We conclude that plasticity has led to the creation of local dialects in the courtship pattern that hampers communication between heterogametic individuals and promotes pre-mating isolation.

Phenotypic plasticity evolves to maximize fitness in variable environments\(^5,6\). It has been proposed that behaviour is an especial aspect of the phenotype and can initiate new directions of evolutionary change because it is labile and can be modified abruptly without the necessity of genetic change\(^2,3\). In isolation, mate choice patterns in populations can evolve independently from each other as a result of random or adaptive
differences in the trajectory of males’ traits and females preferences. As a consequence, the pattern of courtship of males becomes a sign of local identity. Thus the pattern of courtship can be used by females as a signal indicating that males are adapted to a particular environment, and females can increase their fitness by choosing local males to mate with. Because a mistake in the identification of the males' courtship can imply an increase in the cost of reproduction, females are being pressed by sexual selection to mate with the appropriate males. Through frequency-dependent selection, developmental plasticity may generate divergence and contribute to the evolution of reproductive isolation. Male courtship behaviour evolves to capture female attention, thus attracting potential partners and facilitating mating. Courtship can vary between populations within the bounds permitted by the environment. This variation can be the result of 1) selection acting on congenital variants, or 2) phenotypic plasticity, like song or display learning.

*Girardinichthys multiradiatus* is a viviparous fish from Central México with an elaborated courtship pattern and effective female mate choice. In a previous work we found that males of this species showed geographic variation on sexual behaviour, therefore, here we evaluated whether this variation was a consequence of communication failure between heterogametic individuals during courtship. As a preliminary step we re-analysed data published elsewhere and determined that the tendency to perform different elements of the courtship sequence varies predictably between males from five populations. Moreover, the male courtship sequences are associated with their population of origin with substantially greater accuracy than expected by chance (Table 1), suggesting that males of all populations have developed a particular pattern of courtship. We then evaluated whether female choice was responsible for this geographical variation. We recorded female behaviour during courtship, and found that females respond differently to the courtship from heterogametic males than that from their own males (Table 2). Finally we compared the
courtship style of males when encountering females from their own populations with the
courtship they display when facing females from other populations in order to
investigate whether males' courtship sequence was influenced by the behaviour of the
females to which it was directed\textsuperscript{13-15}. We found that males’ courtship to females from
their population significantly differed from the courtship showed to heterogametic
females (Table 3). These results indicate that males are capable of recognizing
homogametic from heterogametic females and adjust their courtship depending on each
females' behaviour.

We suggest that when males court allopatric females they evoke unfamiliar
patterns of female responses, causing males to modify their courtship style. This
behavioural modification demonstrates that courtship in the Amarillo fish is plastic,
even if the adjustment is insufficient to establish appropriate communication with
heterogametic females. Such signal mismatch is characteristic of bird song dialects,
whose origin is unknown\textsuperscript{17}. In the Amarillo fish local variation in courtship style may
be ultimately due to different environmental constraints such as water turbidity or type
of predators\textsuperscript{18}, but may arise through behavioural plasticity which enables fish to adjust
their courtship patterns and become more efficient in their particular environment,
giving rise to population-specific courtship styles.

If males are plastic in their courtship behaviour, why then were they unable to
adjust their behaviour (using the females' feedback) so as to emulate the courtship style
of the female's population? The reason is unlikely to be simply the cost of plasticity\textsuperscript{19,20}
as they did modify their courtship style. What seems to be the case is that both male
courtship style and female responses to this have co-diverged within populations, so that
males encountering unexpected female reactions adjusted their behaviour as they would
have done if a homogametic female had changed her behaviour.\textsuperscript{,} Our data are thus
compatible with the idea that populations of the Amarillo fish have courtship dialects
evidenced by differences in 1) male courtship style, 2) female reactions to male courtship elements, and 3) male reactions to female behavioural feedback. These findings support the idea that phenotypic plasticity is capable of originating reproductive isolation between allopatric populations.

**Methods**

We collected 270 males and 270 females from 5 allopatric populations along the range of distribution of this species\textsuperscript{11}. Fish were taken to the laboratory and placed in 40 l tanks where they were fed with commercial flakes twice a day and were exposed to a 12h light photoperiod. After 15 days fish were separated by sexes and they were placed into individual plastic containers. This procedure not only allowed us to identify the fish individually but as previous work had shown, let us also increase the motivation of the fish to court.

In order to study the geographical variation in the courtship of males, we compared the pattern of displays used by males when courting homogametic and heterogametic females registering frequency and time that males executed behaviours associated with intense courtship (i.e. dynamic display behaviours: fin folding, flagging, and figure of eight), behaviours associated with fins’ exhibition (static display behaviours: lateral fin display and frontal fins display) and copulation attempts\textsuperscript{12}. This procedure was repeated with each combination of populations and each fish was used only in one trial. In order to know the variation in females’ responses, the sequence of displays of males during courtship and the correspondent female responses (vibration, escape, biting, approach to the male, swimming together with the male, no response to the displays), were filmed for 15 minutes. Five homogametic females and five heterogametic females from each population were filmed.
Discriminant analysis. We verified that courtship style is population-specific by performing a canonical discriminant analysis of courtship sequences performed by males to females from their own population and the results were cross validated using the software S-Plus v.2 (MathSoft Inc.). We used population as grouping variable and behavioural displays during courtship as independent variables.

Cochran's Q test. We used Cochran Q test, an extension of McNemar's test for for the significance of changes, that can be used when k (samples) > 2) to analyze female responses to dynamic display behaviours (fin folding, flagging, figure of eight and copulation attempts), and to postural fin displays (lateral display and frontal display) Responses were quantified as 1 (if a given heterogametic female did performed one of the responses shown by homogametic females faced with the same courtship behaviour) or 0 (when the behaviour evoked by the male display was different to that shown by heterogametic females).

1. Coyne, J. A. & Orr, H. A. The evolutionary genetics of speciation. Phil Trans. Roy. Soc. Lond. B 353, 287-305 (1998).
2. West-Eberhard, M. J. Developmental plasticity and the origin of species differences. Proc. Natl. Acad. Sci. USA 102, 6543-6549 (2005).
3. West-Eberhard, M. J. Phenotypic Plasticity and the Origins of Diversity. Annu. Rev. Ecol. Syst. 20, 249-278 (1989).
4. Pomiankowski, A. & Iwasa, Y. What Causes Diversity in Male Sexual Characters. Rev. Suisse Zool. 102, 883-893 (1995).
5. Via, S. et al. Adaptive Phenotypic Plasticity - Consensus and Controversy. Trends Ecol. Evol. 10, 212-217 (1995).
6. Pigliucci, M. Evolution of phenotypic plasticity: where are we going now? Trends Ecol. Evol. 20, 481-486 (2005).
7. Tregenza, T. Divergence and reproductive isolation in the early stages of speciation. *Genetica* **116**, 291-300 (2002).

8. González Zuarth, C. & Macías Garcia, C. Phenotypic differentiation and premating isolation between allopatric populations of *Girardinichthys multiradiatus*. *Proc. R. Soc. Lond. B* **273**, 301-307 (2006).

9. Joshi, A. Inbreeding and sex: canalization, plasticity and sexual selection. *J. Genet.* **84**, 13-15 (2005).

10. Endler, J. A. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76-91 (1980).

11. Endler, J. A. Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vis. Res.* **31**, 587-608 (1991).

12. Lachlan, R. F. & Servedio, M. R. Song learning accelerates allopatric speciation. *Evolution* **58**, 2049-2063 (2004).

13. Macías Garcia, C., Saborio, E. & Berea, C. Does male-biased predation lead to male scarcity in viviparous fish? *J. Fish Biol.* **53**, 104-117 (1998).

14. Coleman, S. W., Patricelli, G. L. & Borgia, G. Variable female preferences drive complex male displays. *Nature* **428**, 742-745 (2004).

15. Smith, C. B. & Martins, E. P. Display plasticity in response to a robotic lizard: Signal matching or song sharing in lizards? *Ethology* **112**, 955-962 (2006).

16. Patricelli, G. L., Coleman, S. W. & Borgia, G. Male satin bowerbirds, *Ptilonorhynchus violaceus*, adjust their display intensity in response to female startling: an experiment with robotic females. *Anim. Behav.* **71**, 49-59 (2006).

17. Olofsson, H. & Servedio, M. R. Sympatry affects the evolution of genetic versus cultural determination of song. *Behav.l Ecol.* **19**, 594-604 (2008).
18  Endler, J. A. Signals, signal condition, and the direction of evolution. *Am. Nat.* 139, s125-s153 (1992).

19.  Dewitt, T. J. Wilson, D. S. Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* 13, 77-81 (1998).

20.  Relyea, R. A. Cost of phenotypic plasticity. *Am. Nat.* 159, 272-282 (2002).

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Table 1. A discriminant analysis based on the tendency to perform dynamic display behaviours classified accurately 40.70% of the males by their population of origin ($F_{10,40} = 2.708$, $p < 0.0001$). Correct assignations are marked with bold characters.

| Population in which males were classified | Porvenir | Salazar | Santiago | San Juanico | Zempoala |
|------------------------------------------|----------|---------|----------|-------------|----------|
| Porvenir                                 | 6        | 0       | 2        | 3           | 1        |
| Salazar                                  | 0        | 5       | 1        | 4           | 0        |
| Santiago                                 | 2        | 1       | 3        | 6           | 0        |
| San Juanico                              | 1        | 4       | 4        | 2           | 1        |
| Zempoala                                 | 0        | 0       | 1        | 1           | 6        |
Table 2. Cochran’s Q tests showed that females differed systematically and significantly in their responses to homogametic and heterogametic males’ courtship. Each row shows the result of comparing the responses of females from each population (i.e. Zempoala in the first row) to heterogametic (Salazar in the first row) to homogametic males.

| Comparisons            | Dynamic display behaviours | Static display behaviours |
|------------------------|----------------------------|---------------------------|
|                        | n(df) | Q     | p   | n(df) | Q     | p   |
| Zempoala Salazar       | 35(1) | 5.00  | 0.025 | 8(1)  | 4.00  | 0.045 |
| Zempoala San Juanico   | 31(1) | 8.00  | 0.005 | 14(1) | 6.00  | 0.01  |
| Zempoala Porvenir      | 31(1) | 11.00 | 0.000 | 14(1) | 4.00  | 0.045 |
| Zempoala Santiago      | 35(1) | 7.00  | 0.014 | 14(1) | 6.00  | 0.014 |
| Salazar Zempoala       | 31(1) | 9.00  | 0.003 | 14(1) | 4.00  | 0.046 |
| Salazar San Juanico    | 35(1) | 10.00 | 0.002 | 14(1) | 6.00  | 0.01  |
| Salazar Porvenir       | 35(1) | 9.00  | 0.003 | 14(1) | 4.00  | 0.046 |
| Salazar Santiago       | 35(1) | 7.00  | 0.008 | 14(1) | 6.00  | 0.01  |
| San Juanico Zempoala   | 31(1) | 12.00 | 0.001 | 14(1) | 4.00  | 0.045 |
| San Juanico Salazar    | 31(1) | 11.00 | 0.001 | 8(1)  | 4.00  | 0.045 |
| San Juanico Porvenir   | 35(1) | 10.00 | 0.002 | 8(1)  | 1.00  | 0.317 |
| San Juanico Santiago   | 35(1) | 15.00 | 0.000 | 14(1) | 3.00  | 0.08  |
| Porvenir Zempoala      | 31(1) | 5.00  | 0.02  | 14(1) | 4.00  | 0.045 |
| Porvenir Salazar       | 35(1) | 10.00 | 0.002 | 6(1)  | n.c   | n.c   |
| Porvenir San Juanico   | 31(1) | 5.00  | 0.02  | 14(1) | 4.00  | 0.045 |
| Location              | Total 1 | Total 2 | Total 3 | Total 4 | Total 5 |
|-----------------------|---------|---------|---------|---------|---------|
| Porvenir Santiago     | 35(1)   | 7.00    | 0.008   | 14(1)   | 3.00    | 0.08    |
| Santiago Zempoala     | 31(1)   | 6.00    | 0.01    | 14(1)   | 1.00    | 0.317   |
| Santiago Salazar      | 35(1)   | 7.00    | 0.058   | 14(1)   | 1.00    | 0.317   |
| Santiago San Juanico  | 31(1)   | 7.00    | 0.008   | 14(1)   | 3.00    | 0.083   |
| Santiago Porvenir     | 35(1)   | 8.00    | 0.005   | 8(1)    | 3.00    | 0.083   |
Table 3. Planned comparisons showed that males modified their courtship pattern when they were exposed to heterogametic females.

| Males population | Females population | F         | p     |
|------------------|--------------------|-----------|-------|
| Porvenir         | Salazar            | $F_{(4,45)}=11.560$ | 0.001 |
| Porvenir         | Santiago           | $F_{(4,45)}=5.841$  | 0.019 |
| Porvenir         | San Juanico        | $F_{(4,45)}=17.586$ | 0.000 |
| Porvenir         | Zempoala           | $F_{(4,45)}=7.754$  | 0.007 |
| Salazar          | Porvenir           | $F_{(4,36)}=1.288$  | 0.264 |
| Salazar          | Santiago           | $F_{(4,36)}=4.387$  | 0.043 |
| Salazar          | San Juanico        | $F_{(4,36)}=3.302$  | 0.077 |
| Salazar          | Zempoala           | $F_{(4,36)}=5.979$  | 0.019 |
| Santiago         | Porvenir           | $F_{(4,48)}=5.434$  | 0.024 |
| Santiago         | Salazar            | $F_{(4,48)}=4.184$  | 0.046 |
| Santiago         | San Juanico        | $F_{(4,48)}=12.428$ | 0.000 |
| Santiago         | Zempoala           | $F_{(4,48)}=0.609$  | 0.439 |
| San Juanico      | Porvenir           | $F_{(4,44)}=0.098$  | 0.755 |
| San Juanico      | Salazar            | $F_{(4,44)}=4.822$  | 0.033 |
| San Juanico      | Santiago           | $F_{(4,44)}=1.933$  | 0.171 |
| San Juanico      | Zempoala           | $F_{(4,44)}=4.201$  | 0.045 |
| Zempoala         | Porvenir           | $F_{(4,27)}=3.903$  | 0.058 |
| Zempoala         | Salazar            | $F_{(4,27)}=7.022$  | 0.013 |
| Location       | Location       | F(4,27) | p-value |
|----------------|----------------|---------|---------|
| Zempoala       | Santiago       | 5.418   | 0.027   |
| Zempoala       | San Juanico    | 7.850   | 0.009   |
