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Rapid molecular sexing of three-spined sticklebacks, *Gasterosteus aculeatus* L., based on large Y-chromosomal insertions

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Abstract There is a need for rapid and reliable molecular sexing of three-spined sticklebacks, *Gasterosteus aculeatus*, the supermodel species for evolutionary biology. A DNA region at the 5′ end of the sex-linked microsatellite Gac4202 was sequenced for the X chromosome of six females and the Y chromosome of five males from three populations. The Y chromosome contained two large insertions, which did not recombine with the phenotype of sex in a cross of 322 individuals. Genetic variation (SNPs and indels) within the insertions was smaller than on flanking DNA sequences. Three molecular PCR-based sex tests were developed, in which the first, the second or both insertions were covered. In five European populations (from DE, CH, NL, GB) of three-spined sticklebacks, tests with both insertions combined showed two clearly separated bands on agarose minigels in males and one band in females. The tests with the separate insertions gave similar results. Thus, the new molecular sexing method gave rapid and reliable results for sexing three-spined sticklebacks and is an improvement and/or alternative to existing methods.

Keywords Fish · PCR-based sex test · Population · Sex determination

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Introduction

Differences in ecology, physiology, morphology and behaviour between the sexes are described in many animal species (e.g. McPherson and Chenoweth 2012), raising the need for taking sex into account in scientific studies. However, sexing of animal species on the basis of non-invasive phenotypic traits is often not possible or time consuming, e.g. in early life stages or as adults in sexually monomorphic species. Molecular sexing methods have the advantage of overcoming these restrictions of phenotypic sexing. They are frequently applied in birds and mammals, which have relatively conserved genetic sex-determination systems (Bachtrog et al. 2014). Molecular sexing in birds is applied in a plethora of research areas, ranging from population, behaviour and evolutionary studies to sex ratio evolution and species mating system assessment, improvement of captive breeding programmes, managing of wildlife species, analysis of breeding strategies in commercial poultry to forensic studies (Morinha et al. 2012). In fishes, molecular sexing is less frequently applied, probably because they show diverse sex-determination systems (Bachtrog et al. 2014).

The three-spined stickleback, *Gasterosteus aculeatus* L., is a small teleost fish species that has been extensively studied from a behavioural and ecological perspective (Wootton 1976, 1984; Bell and Foster 1994; Östlund-Nilsson et al. 2007).

Since the integration of molecular genetics in stickleback research (Peichel et al. 2001), the three-spined stickleback is recognised as a supermodel species for evolutionary biology (Gibson 2005; Barber and Nettleship 2010). Three-spined sticklebacks show a distinctive sexual dichromatism during the reproductive season (Wootton 1976, 1984), whereas they appear monomorphic outside the breeding season (Wootton 1976, 1984). Sex differences of non-reproductive fish have been described for example with respect to boldness (King et al. 2013), migratory behaviour (Cano et al. 2008), feeding mechanism (McGee and Wainwright 2013) and parasite load (Arnold et al. 2003). However, many sex differences in stickleback's behaviour and ecology might remain elusive due to the lack of a simple, rapid and reliable sexing method. *Gasterosteus aculeatus* has mostly a XY sex-determination system, with linkage group 19 (LG19) being the sex chromosome pair (Peichel et al. 2004; Ross and Peichel 2008; Urton et al. 2011). Three molecular sex tests for *G. aculeatus* have been developed. Griffiths et al. (2000) assessed sex-linked AFLP markers, while Peichel et al. (2004) found a sex-specific polymorphism in the 3′UTR of the isocitrate dehydrogenase (*Idh*) gene at LG19. Toli et al. (2016) developed a multi-marker assay based on a Bayesian update approach using genotype scores from three sex-linked loci, i.e. the *Idh* (Peichel et al. 2004), *Gasm6* (Natri et al. 2013) and *Stn190* loci (Peichel et al. 2001). Potentially, other tests may be developed from sex-specific microsatellites established by Peichel et al. (2001), Shikano et al. (2011b), Shimada et al. (2011) and Natri et al. (2013). The current molecular assays are, however, not perfect (see Discussion) and, thus, an alternative would be useful.

In the present study, we developed a new, simple, rapid and reliable molecular sex test for *G. aculeatus* and tested its applicability for various European populations.

Materials and methods

Study populations and species

An unpublished (Largiadèr et al.) sex-linked microsatellite Gac4202 (repeat $(GA)_{25}$, primer pair Alt-1 and Alt-2 (Table 1) was developed from a wild-caught fish of a three-spined stickleback population near Roche/Montreux, Switzerland (46° 26′ N, 6° 55′ E) (see Largiadèr et al. 1999 for details and procedure for microsatellite development). Variability at Gac4202 was tested using wild-caught three-spined stickleback fish of the Roche population, lab-bred F1 fish from wild-caught parents of a population from the Wohlensee (near Bern, Switzerland, 46° 57′ N, 7° 28′ E) (see Mazzi and Bakker 2003 for standardised conditions of rearing and maintenance) and wild-caught fish from a Dutch anadromous population on the island of Texel (53° 03′ N, 4° 48′ E).

Based on the complete 850-bp-long sequence of the microsatellite clone Gac4202, which was derived from a female fish, a molecular sex test was developed by amplifying a sex chromosome specific length polymorphism using the N1-1 and Alt-1REV primers (Table 1), i.e. a ca. 271-bp-long fragment on the X chromosome and a ca. 813-bp-long fragment on the Y chromosome, respectively.

The generality of the applicability of the sex test was tested with fresh or 97% ethanol samples of adult wild-caught fish from the following European three-spined stickleback populations: a pond population from Euskirchen near Bonn, Germany (50° 38′ N, 6° 47′ E), an anadromous population from the island of Texel, the Netherlands (53° 3′ N, 4° 48′ E) and a population from the slightly acid lake Loch Tormasad on the island of North Uist, Hebrides, Scotland (57° 33′ N, 7° 19′ W).

In addition to molecular sexing, the sex of all adult fishes was determined by inspection of the gonads.

Sequencing

Genomic DNA was prepared from muscle tissue following a phenol-chloroform extraction method (Sambrook et al. 1989). Primers used for the amplification of a region at the 5′ end of the Gac4202 microsatellite that included two insertions on the Y chromosome were N1-1 and Alt-1REV (Table 1). PCR products of the X and Y chromosomal fragments were cloned using the Advantage PCR Cloning Kit (Clontech), following the instructions of the manufacturer. Sequencing of cloned PCR fragments encompassing the Y- and X- chromosomal amplification products of several females and males of the three above-mentioned populations (Roche, Wohlensee and Texel) was done in order to estimate the degree of sequence conservation in this region. Cloning and DNA sequencing was done following Largiadèr et al. (1999) and Heckel et al. (2002).

Sex test

Genomic DNA was prepared from whole animals (juveniles) or dorsal spines (adults) according to a Chelex resin (Bio-Rad) extraction protocol (Estoup et al. 1993). PCR amplifications were done in a 10 μL volume using a thermocycler (T1 Thermoblock, Biometra). Each reaction contained 2 μL DNA extraction, 0.3 μL of each primer (10 pmol/μL), 0.05 μL Taq polymerase (5 u/μL), 0.2 μL dNTPs (10 mM), 1 μL buffer YS (10×), 2.0 μL Enhancer Sol P and 4.15 μL distilled water. The reaction conditions were: initial denaturation at 94 °C for 5 min, followed by 30 cycles consisting of 30 s at 94 °C, 30 s at 57 °C and 1 min at 72 °C, then a final extension cycle of 10 min at 72 °C, then cooled down at 12 °C. Amplification products were separated and made visible with ethidium bromide on 1.5% or 2% mini agarose gels, together with a DNA size standard (GeneRuler 50 bp or 100 bp DNA Ladder, Fermentas). Exact allele sizes were determined using a CEQ 8800 or CEQ 8000

Table 1 Sequences of the primer pairs (F: forward, R: reverse) to amplify insertion 1, insertion 2, insertion 1 + 2 and the microsatellite Gac4202

Primer pair name	Primer sequence 5′-3′	Amplified region	Position*
Alt-1	F: CGGGGCGATGGCAACGAC	Microsatellite Gac4202	1130–1147
Alt-2	R: CACGCGCCCTTTCCACTCAG		1300–1319
N1-1	F: CATTACAGAAGATGCATTGTTCAG	Y-chromosomal insertion 1 + 2	330–353
Alt-1REV	R: GTCGTTGCCATCGCCCCG		1130–1147
N1-1	F: CATTACAGAAGATGCATTGTTCAG	Y-chromosomal insertion 1	330–353
N2-1REV	R: ATCTCTGACACTCACAGGTG		809–828
N2-1	F: ACCTGTGAGTGTCAGAGATG	Y-chromosomal insertion 2	810–829
Alt-1REV	R: GTCGTTGCCATCGCCCCG		1130–1147

*Position of primer sequence in the alignment given in supplementary Fig. S1

capillary sequencer (Beckman-Coulter® GmbH) and 400 bp (Kit-400, AB Sciex) and 1000 bp (MapMarker 1000, BioVentures) sizing standards. Three sex tests were performed (Table 1): one covering both insertions on the Y chromosome (primer pair N1-1 and Alt-1REV), one covering insertion 1 (primer pair N1-1 and N2-1REV) and one covering insertion 2 (primer pair N2-1 and Alt-1REV).

Results

Microsatellite Gac4202

In the Roche and Wohlensee populations, the microsatellite Gac4202 showed a sex-linked inheritance: the Y-chromosomal band was invariably 185 bp long, while there was allelic variation on the X chromosome: 164 and 166 bp (and a null allele as deduced from a missing band on the X chromosome in some males and females) in the Roche population and 164, 170 and 172 bp in the Wohlensee population. So, males and females could be distinguished on the basis of the presence or absence of the longer Y-chromosomal band. The sex-linked XY inheritance was fully confirmed by comparing allele sizes at Gac4202 of adult progeny (163 females, 159 males; sex also determined by dissection) and their parents (24 females, 16 males) of the Wohlensee population. On average, 13.4 progeny (range 8–23) per cross were analysed. In the anadromous, genetically heterogeneous Texel population (Heckel et al. 2002), there existed more than 20 alleles on both the X and Y chromosome, with great overlap in allele sizes between the chromosomes. The complex band pattern did not allow sex identification on the basis of Gac4202. We, therefore, characterised a region at the 5′ end of Gac4202 in order to develop a more universal sex test.

Y-chromosome insertions

Sequencing a region at the 5′ end of Gac4202 revealed two insertions on the Y chromosome of males from the Roche, Wohlensee and Texel populations (Figs 1 and 2, supplementary Fig. S1) compared to the X chromosome. The insertion closest to Gac4202 (called insertion 2) measured 238 bp, followed by a 89-bp-long region on both the Y and X chromosomes, and a second larger insertion (called insertion 1) of 307 bp. The insertions are well conserved, showing low variation among the ten cloned Y chromosomal fragments of fish from three populations (SNPs and indels at maximal 13.2% of the positions, excluding polymorphisms based on ambiguous nucleotides: 6.4%; supplementary Fig. S1). The 12 cloned X-chromosomal fragments and corresponding regions on the ten Y chromosomes were significantly more variable, showing SNPs and indels at maximal 30.7% (minimum when excluding ambiguous nucleotides: 18.2%) of the positions (supplementary Fig. S1) (difference in SNP and indel frequency between inserts and others regions: G test, $G = 30.3$, $df = 1$, $p < 0.001$, and $G = 22.7$, $df = 1$, $p < 0.001$, respectively). SNPs and indels were present between populations and between sexes and also among individuals within populations. Finally, sequence variation was even detected among cloned fragments of the same individual, which can be explained by in vitro errors of the polymerase during PCR amplification. Thus, the observed sequence variation probably represents an overestimation. We can not, however, exclude the possibility that this intra-individual sequence variability may also point to the existence of multiple copies of these fragments in the genome.

Blast searches (Zhang et al. 2000) indicated high sequence similarity with a *G. aculeatus* whole genome shotgun sequence (AANH01001637.1; contig no. 1.001636) (Fig. 1, supplementary Fig. S1). This sequence is part of a draft stickleback genome (Jones et al. 2012) and is located on chromosome 19, which was identified as the X chromosome. Interestingly, the sequence has been derived from a single female individual (Jones et al. 2012), it lacks insertion 1 but not insertion 2, which is 3 bp longer (241 bp instead of 238 bp) than that observed for all cloned Y-chromosomal PCR fragments (Fig. 1, supplementary Fig. S1).

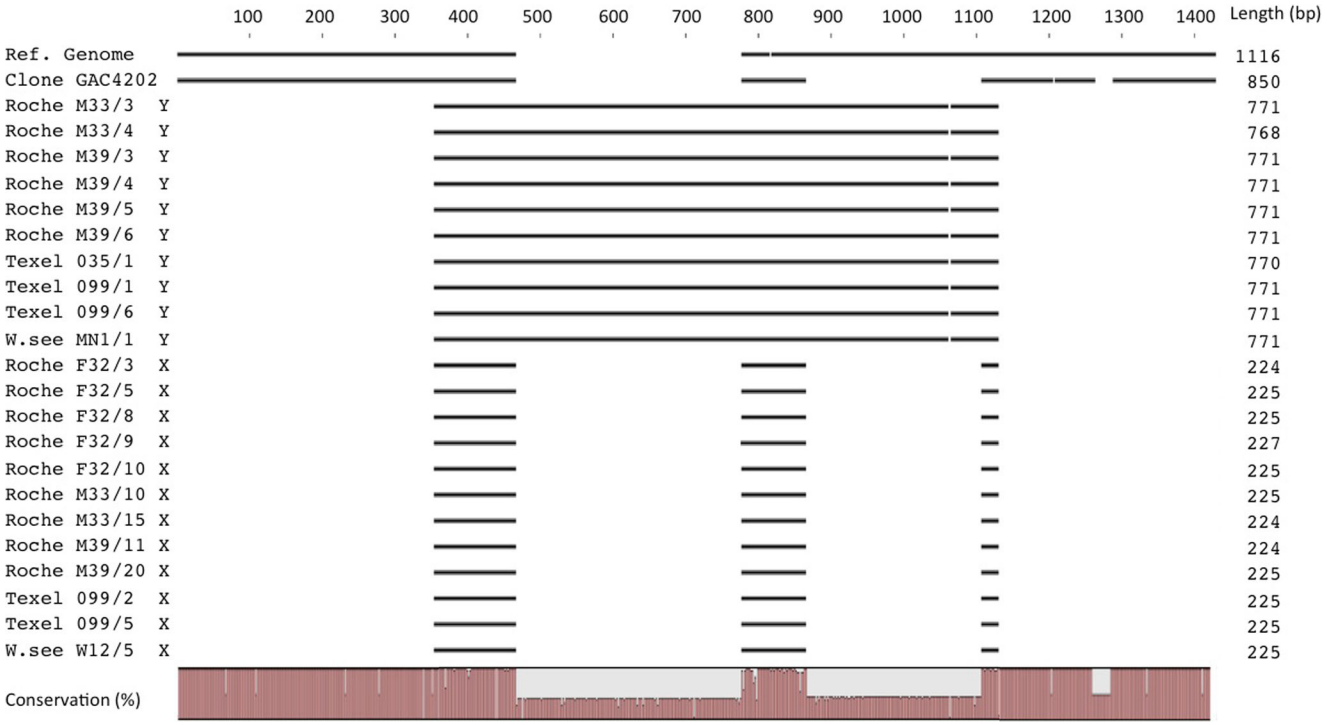

Fig. 1 Schematic representation of the sequence alignment of the cloned Y- and X- chromosomal PCR fragments with the *Gasterosteus aculeatus* (accession number: AANH01001637.1) whole genome shotgun sequence contig no. 1.001636 and the microsatellite clone Gac4202 sequence. See supplementary Fig. S1 for a detailed alignment

The Y-chromosomal insertions offered simple molecular sex tests that produced two clearly separated bands in males and one band in females. Amplifying insertion 1 + 2 produced a ca. 271-bp band in females and both a 271-bp and a ca. 813-bp band in males, amplifying insertion 1 produced a ca. 190-bp band in females and both a ca. 190-bp and a ca. 497-bp band in males and amplifying insertion 2 produced a ca. 100-bp band in females and both a ca. 100-bp and a ca. 338-bp band in males (Figs 1, 2 and 3).

Applicability of sex test

In all five European three-spined stickleback populations, the sex test involving insertions 1 and 2 gave unambiguous results: two bands in males and one band in females (Table 2, Fig. 3). There existed some variation in allele size within and between populations on the Y chromosome and, to a more limited extent, on the X chromosome (Table 2).

Discussion

Crosses of three-spined sticklebacks from the Swiss Wohlensee population with different alleles at the microsatellite Gac4202 confirmed the XY sex-determination system that had been characterised by Peichel and co-workers (Peichel et al. 2004; Ross and Peichel 2008; Urton et al. 2011). By sequencing the 5′ end of the sex-linked microsatellite Gac4202, we found two insertions of 238 bp and 307 bp, respectively, separated by 89 bp, on the Y chromosome of

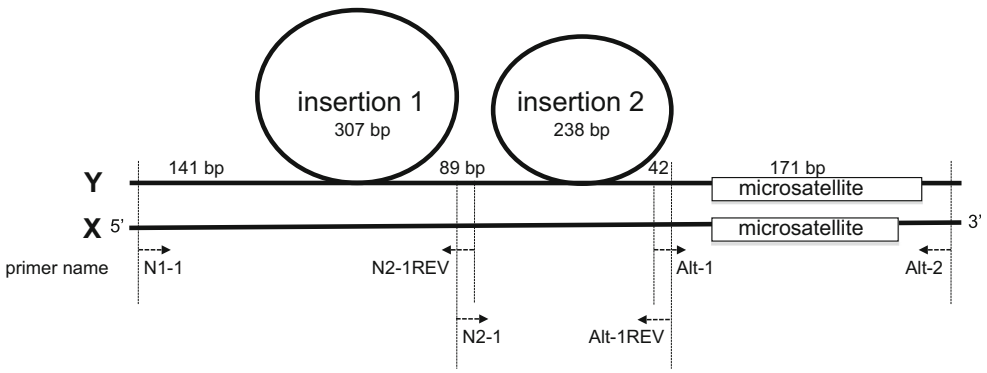

Fig. 2 Schematic representation of two insertions on the Y chromosome at the 5′ end of the Gac4202 sex-linked microsatellite in *G. aculeatus*. Indicated are sizes in bp, as well as the name of the primers used to amplify insertion 1, insertion 2, insertion 1 + 2 and the microsatellite

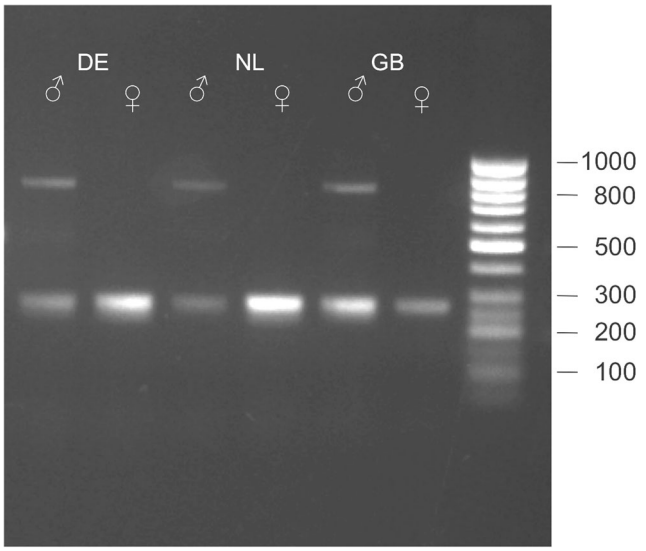

Fig. 3 Part of ethidium bromide stained agarose gel showing an example of sex tests based on insertion 1 + 2 with males and females from various European three-spined stickleback populations. DE: German population (Euskirchen), NL: Dutch population (Texel) and GB: Scottish population (Loch Tormasad). Each lane represents a single sample. ♂ and ♀ indicate male and female, respectively. The far right lane shows a 1000 bp size standard

the three-spined stickleback that were absent on the X chromosome (see also Peichel et al. 2004). The sequences obtained from this study mapped to the X chromosome 19 of the three-spined stickleback draft genome (Jones et al. 2012) in an apparently non-coding region. In comparison to our X- and Y-chromosomal sequences, the reference genome, which had been derived from a single female stickleback from Alaska (Jones et al. 2012), had a chimeric organisation possessing one of the presumably Y-chromosome specific introns. This may indicate a recombination event between the Y and X chromosomes in this population. Alternatively, there could be an insertion on the Y chromosome that is unique to the European populations sampled.

Amplification of the region containing the two insertions yielded a very reliable molecular sex test in different populations: in over 50 fish tested of six stickleback populations, males yielded two well-separated bands, while females yielded one. The sexing was done easily and rapidly by PCR amplification of DNA of this region, followed by separation on an agarose minigel.

Our test adds to the molecular sexing tests in the three-spined stickleback. Molecular sexing is a powerful tool and has been applied in various research areas using sticklebacks, such as parasitology (Arnold et al. 2003), ecotoxicology (Hahlbeck et al. 2004; Bernhardt et al. 2006), ontogeny (Lewis et al. 2008), genetics (Stärner et al. 2004), endocrinology (Bell et al. 2007) and evolutionary biology (Lenz et al. 2009; Bell et al. 2011; Leinonen et al. 2011a, b; Loehr et al. 2012; Ramler et al. 2014).

Also, for the nine-spined stickleback, *Pungitius pungitius*, which is emerging as another stickleback model for evolutionary biology, genetic and behavioural research (Merilä 2013), a reliable sex-specific microsatellite has previously been developed (Shikano et al. 2011a). It had been applied by Shikano and Merilä (2011) in a comparative study on body size and vertebrae number.

Sexing was 100% reliable with our sex test (this study) and is, thus, more reliable than the molecular sex tests that had been developed for the three-spined stickleback thus far. Griffiths et al. (2000) also reached 100% reliability of their test in 53 fish from three three-spined stickleback populations. However, in a study of a Swedish population, the control band that should show up in both sexes proved not to be reliable, with variable reliability between broods (Hahlbeck et al. 2004). Males were, therefore, much more reliably identified than females with the Griffiths et al. (2000) test in that population. The average reliability of the sex test of Peichel et al. (2004) as assessed with nearly 400 fish from crosses between ecotypes was higher than 99% (error rate in one cross 1.52% and in the other cross 0.61%). In Toli et al. (2016), the error rate for the *Idh* marker varied greatly, that is, between 0% and 51%, depending on the population and scorer. Per population, about 50 fish were sexed and the agarose gels judged by four scorers. The error rate of the other markers was much lower: on average, 0% and

Table 2 Amplification products (number of bands separated by 200 bp or more and allele sizes) made visible on agarose gels of DNA from males (m) and females (f) of various European populations of *Gasterosteus aculeatus*. Primers used were developed for insertion 1 (1), insertion 2 (2) or insertion 1 + 2 (1 + 2) at the 5′ end of Gac4202 in *G. aculeatus*

Population	Sex	No. of fish	No. of bands 1	Allele sizes of 1	No. of bands 2	Allele sizes of 2	No. of bands 1 + 2	Allele sizes of 1 + 2
Texel, NL	m	5	2	197, 494	2	95, 335	2	274, 838/840
Texel, NL	f	5	1	197	1	95	1	274
Euskirchen, DE	m	5	2	195/197, 494/496	2	95, 335	2	274, 836/838
Euskirchen, DE	f	5	1	197	1	95	1	274
Tormasad, GB	m	4	2	197, 494	2	95, 335	2	274, 836/838/840
Tormasad, GB	f	5	1	197	1	95	1	274

1.7% for the *Gasm6* and *Stn190* markers, respectively (Toli et al. 2016). In order to minimise errors of sex identification, Toli et al. (2016) strongly advocate a multi-marker approach. The errors with these different markers could be due to several factors, such as the phenotypic sex identification was incorrect, the marker recombined with the sex determination locus, different populations do not share the same X and Y alleles or the genetic marker is unreliable. Our sex identification test has the advantage that bands are far apart (more than 200 bp), thereby minimising inter-scorer variability and user error. The presence of one of the Y-chromosomal insertions in the reference genome suggest that our tests involving insertion 2 may not be applicable to three-spined sticklebacks of all three evolutionary lineages (i.e. Pacific, Atlantic, Japanese Sea; Toli et al. 2016). Future research has to verify this.

Significantly fewer SNPs and indels were assessed within than outside the insertions for unknown reasons. An open question is the source and function of the inserted sequences, but it cannot be excluded that the variation is partly due to errors of the polymerase during PCR amplification. All investigated males had the large insertions on the Y chromosome, while these were lacking on all investigated X chromosomes, so recombination seems to be suppressed.

Sample sizes in the present study were too low to completely comprise allelic variation at the loci used for the sex tests. The results point to the presence of limited intra- and inter-populational variation that should be further explored in future studies.

DNA for the sex test was extracted from spines of adult fish. Spine clipping is a common way to mark three-spined sticklebacks both in the laboratory and in the field (e.g. Bakker and Mundwiler 1994). It has been shown not to reduce survival and only temporarily increases immune responses (Wedekind and Little 2004; Henrich et al. 2014). Therefore, the new sex test is not only applicable in dead specimens, but can also be used in live fish.

To sum up, this study provides a cheap, easy and highly reliable way to determine the sex of three-spined sticklebacks of varying age and of different European populations. The sex identification test is an improvement and/or alternative to existing methods (Griffiths et al. 2000; Peichel et al. 2004; Toli et al. 2016). Thus, it gives researchers working on this supermodel a useful tool to include sex as an explaining variable in their studies.

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Author contributions TCMB and CRL designed the research; TCMB and CRL obtained funding for the study; TG and JGF conducted the experimental work; TCMB, TG and CRL analysed the data; TCMB wrote the manuscript and was supported by CRL and JGF.

Compliance with ethical standards The study conforms to the Association for the Study of Animal Behaviour guidelines for the use of animals in research as well as to the legal requirements of Switzerland and Germany.

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Conflict of interest The authors declare that they have no conflict of interest.

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Online Resource 1

Rapid molecular sexing of three-spined sticklebacks, *Gasterosteus aculeatus* L., based on large Y-chromosomal insertions

Journal of Applied Genetics

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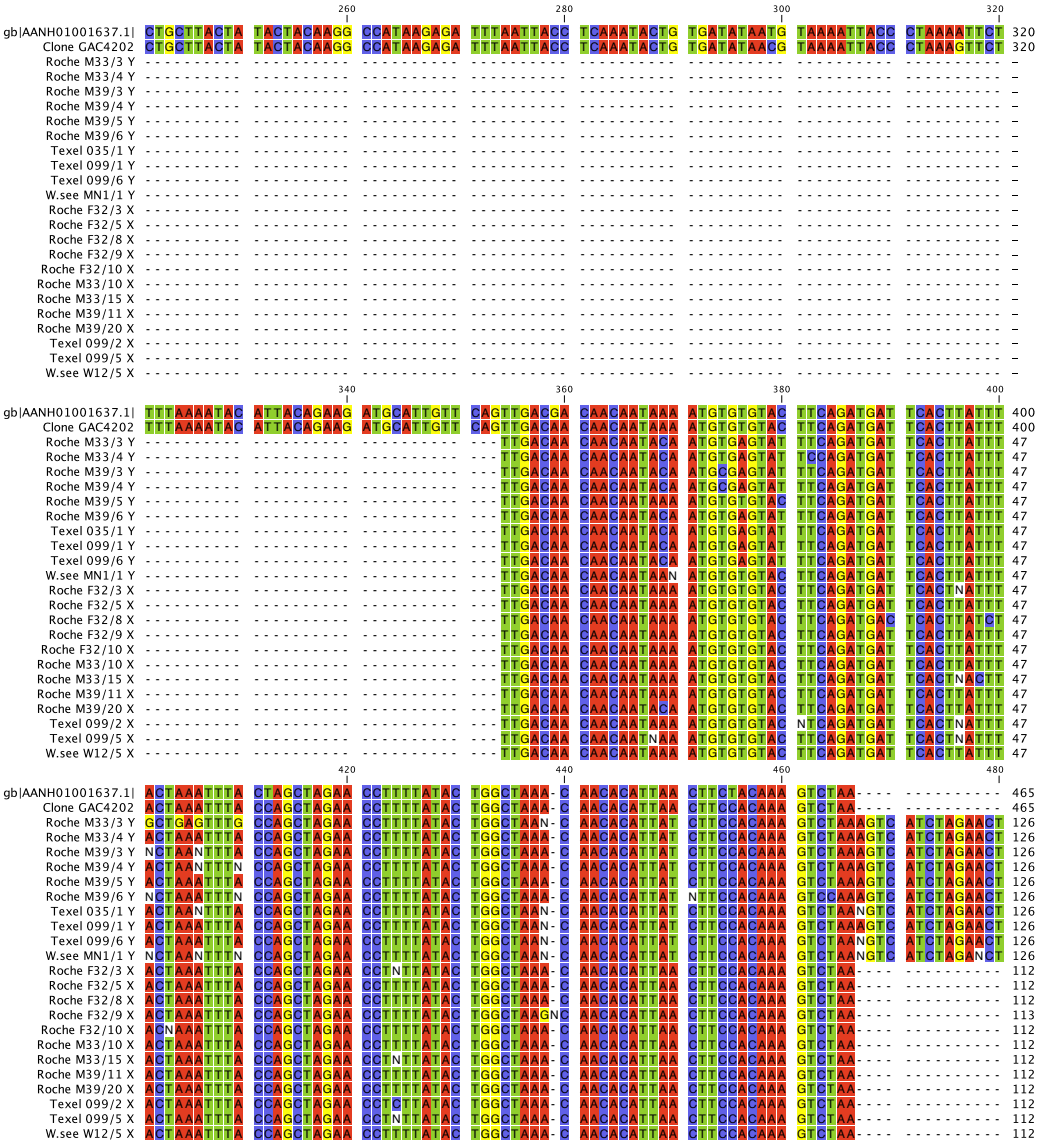
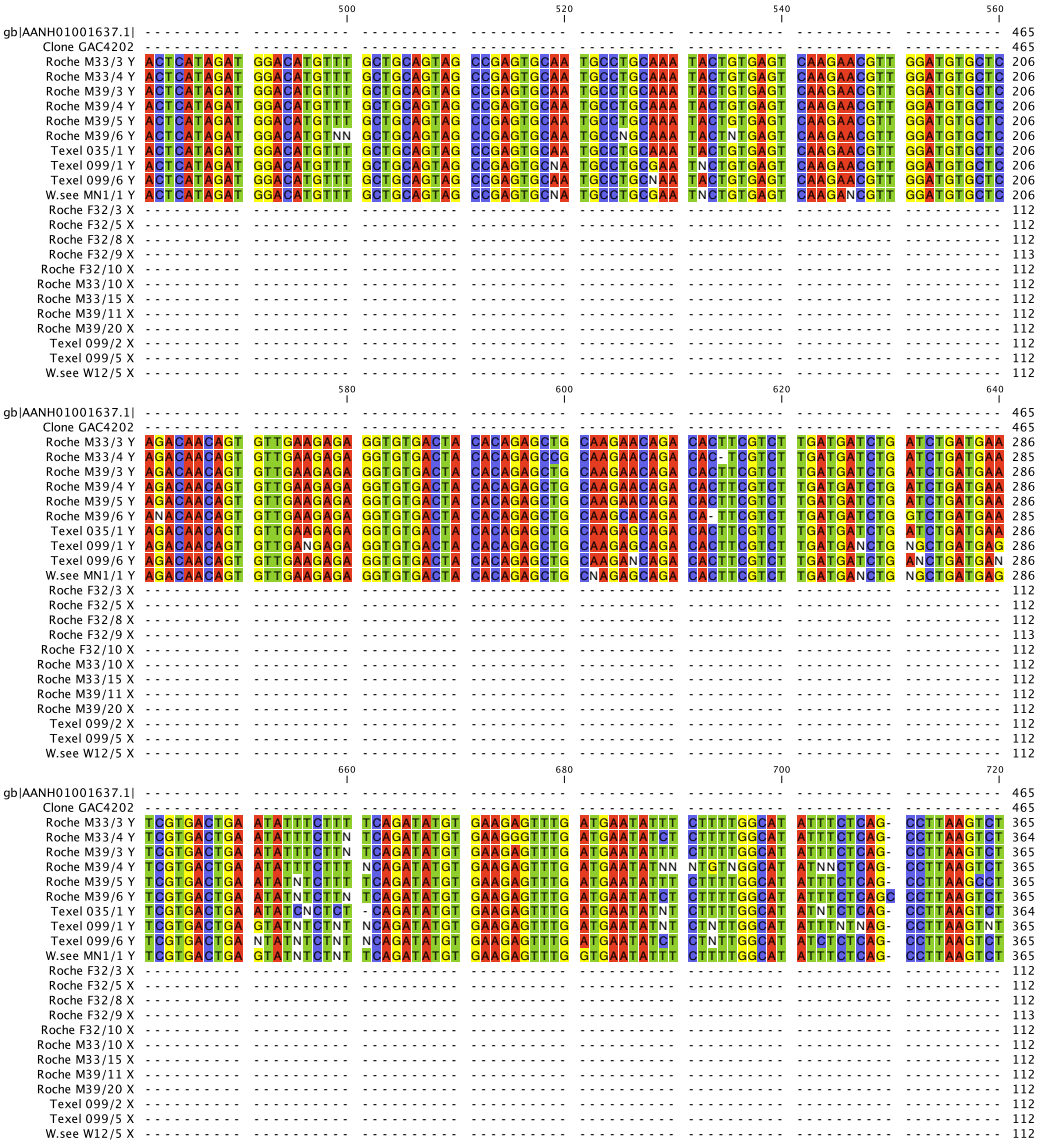
Fig. S1 Sequence alignment of the cloned Y- and X- chromosomal PCR-fragments with the *Gasterosteus aculeatus* (accession number: AANH01001637.1) whole genome shotgun sequence contig no. 1.001636 and the microsatellite clone GAC4202 sequence. Cloned fragments are aligned without primer sequences. Of the ca. 10,000 assessed nucleotides 1.5% could not be unambiguously determined (indicated by N). Labels for cloned fragments are given as:
"Population"_"Individual"/"Number of clone"/"Y or X chromosomal fragment"

-: indicates deletion (or insertion at this position in other fish)


gb|AANH01001637.1|
Clone GAC4202
Roche M33/3 Y
Roche M33/4 Y
Roche M39/3 Y
Roche M39/4 Y
Roche M39/5 Y
Roche M39/6 Y
Texel 035/1 Y
Texel 099/1 Y
Texel 099/6 Y
W.see MN1/1 Y
Roche F32/3 X
Roche F32/5 X
Roche F32/8 X
Roche F32/9 X
Roche F32/10 X
Roche M33/10 X
Roche M33/15 X
Roche M39/11 X
Roche M39/20 X
Texel 099/2 X
Texel 099/5 X
W.see W12/5 X


gb|AANH01001637.1|
Clone GAC4202
Roche M33/3 Y
Roche M33/4 Y
Roche M39/3 Y
Roche M39/4 Y
Roche M39/5 Y
Roche M39/6 Y
Texel 035/1 Y
Texel 099/1 Y
Texel 099/6 Y
W.see MN1/1 Y
Roche F32/3 X
Roche F32/5 X
Roche F32/8 X
Roche F32/9 X
Roche F32/10 X
Roche M33/10 X
Roche M33/15 X
Roche M39/11 X
Roche M39/20 X
Texel 099/2 X
Texel 099/5 X
W.see W12/5 X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520
540
560
gb|AANH01001637.1|
Clone GAC4202
Roche M33/3 Y
Roche M33/4 Y
Roche M39/3 Y
Roche M39/4 Y
Roche M39/5 Y
Roche M39/6 Y
Texel 035/1 Y
Texel 099/1 Y
Texel 099/6 Y
W.see MN1/1 Y
Roche F32/3 X
Roche F32/5 X
Roche F32/8 X
Roche F32/9 X
Roche F32/10 X
Roche M33/10 X
Roche M33/15 X
Roche M39/11 X
Roche M39/20 X
Texel 099/2 X
Texel 099/5 X
W.see W12/5 X
580
600
620
640
660
680
700
720


740
760
780
800
gb|AANH01001637.1|
Clone GAC4202
Roche M33/3 Y
Roche M33/4 Y
Roche M39/3 Y
Roche M39/4 Y
Roche M39/5 Y
Roche M39/6 Y
Texel 035/1 Y
Texel 099/1 Y
Texel 099/6 Y
W.see MN1/1 Y
Roche F32/3 X
Roche F32/5 X
Roche F32/8 X
Roche F32/9 X
Roche F32/10 X
Roche M33/10 X
Roche M33/15 X
Roche M39/11 X
Roche M39/20 X
Texel 099/2 X
Texel 099/5 X
W.see W12/5 X
820
840
860
880
900
920
940
960


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Clone GAC4202 554
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Roche M39/4 Y CCTCCAGTTC CGTCTTTGGA CGCCTCCGAG CCTTGTTGAC AGCGTGGTGA AGACATCTAT GTGAGCCATA TGAAAACACT 685
Roche M39/5 Y CCTCCAGTTC CGTCTTTGGA CGCCTCCGAG CCTTGTTGAC AGCGTGGTGA AGACATCTAT GTGAGCCATA TGAAAACACT 685
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Texel 035/1 Y CCTCCAGTTC CGTCTTTGTA CGCCTCCGAG CCTTGTTGAC AGCGTGGTGA GGACATCTAT GTGAGCCATA TGAAAACACT 684
Texel 099/1 Y CCTCCAGTTC CGTCTTTGGA CGCCTCCGAG CCTTGTTGAC AGCGTGGTGA AGACATCTAT GTGAGCCATA TGAAAACACT 685
Texel 099/6 Y CCTCCAGTTC CGTCTTTGGA CGCCTCCGAG CCTNGTTGAC AGCGTGGTGA AGACANCTAT GTGAGCCATA TGAAAACACT 685
W.see MN1/1 Y CCTCCAGTTC CGTCTTTGGA CGCCTCCGAG CCTTGTTGAC AGCGTGGTGA AGACATCTAT GTGGGCCATA TGAAAACACT 685
Roche F32/3 X 200
Roche F32/5 X 201
Roche F32/8 X 201
Roche F32/9 X 203
Roche F32/10 X 201
Roche M33/10 X 201
Roche M33/15 X 200
Roche M39/11 X 200
Roche M39/20 X 201
Texel 099/2 X 201
Texel 099/5 X 201
W.see W12/5 X 201
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Clone GAC4202 GACAG CACTAACAAG 569
Roche M33/3 Y GTGCATCCTC CTCCTCCTCC ---GTCATCC TCTGGCGTGT CCTTGCTGAG CTTGACCTGC TCAGAGACAG CACTAACAAG 762
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Roche M39/5 Y GTGCATCCTC CTCCTCCTCC ---GTCATCC TCTGGCGTGT CCTTGCTGAG CTTGACCTGC TCAGAGACAG CACTAACAAG 762
Roche M39/6 Y GTGCATCCTC CTCCTCCTCC ---GTCATCC TCTGGCGTGT CCTTGCTGAG CTTGACCTGC TCAGAGACAG CACTAACAAG 762
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Texel 099/6 Y GTGCATCCTC CTCCTCCTCC ---GTCATCC TCTGGCGTGT CCTTGCTGAG CTTGATCTGC TCAGAGACAG CACTAACAAG 762
W.see MN1/1 Y GTGCATCCTC CTCCTCCTCC ---GTCATCC TCTGGCGTGT CCTTGCTGAG CTTGACCTGC TCAGAGACAG CACTAACAAG 762
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Roche M39/11 X GACAG CACTAACAAG 215
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Texel 099/5 X GACAG CACTAACAAG 216
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Roche M33/4 Y CCA-AACCA- 768
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Texel 099/6 Y CCAGAACCA- 771
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Roche F32/9 X CCAGAACCA- 227
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Roche M39/20 X CCAGAACCA- 225
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