Exploring Jordan’s rule in Pacific three-spined stickleback

Gasterosteus aculeatus

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Coastal marine Gasterosteus aculeatus were captured from seven locations along the Pacific coast of North America, ranging across 21·8° latitude to test Jordan’s rule, i.e. that vertebral number should increase with increasing latitude for related populations of fish. Vertebral number significantly increased with increasing latitude for both total and caudal vertebral number. Increasing length with latitude (sensu Bergmann’s rule) was also supported, but the predictions for Jordan’s rule held when controlling for standard length. Pleomerism was weakly evidenced. Gasterosteus aculeatus exhibited sexual dimorphism for Jordan’s rule, with both sexes having more vertebrae at higher latitudes, but only males showing a positive association between latitude and the ratio of caudal to abdominal vertebrae. The number of dorsal- and anal-fin rays and basals increased with increasing latitude, while pectoral-fin ray number decreased. This study reinforces the association between phenotypic variation and environmental variation in marine populations of G. aculeatus.

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Key words: adaptation; cline; ecogeography; plasticity; pleomerism; temperature.

INTRODUCTION

Linking genetic variation to phenotypic and environmental variation is key to elucidating the evolutionary processes responsible for diversity. Typically, this is addressed under an hypothesis-testing framework, such as common environment or reciprocal transplant experiments. Alternatively, researchers have sought out rules that link phenotypic and environmental variation as a means of explaining process through pattern.

Ecogeographic rules, based upon patterns of phenotypic variation along some geographic continuum, were originally formulated as compelling examples of adaptation. Desert animals with larger ears or polar animals with thicker fur than temperate conspecifics provided early evidence that selection could modify phenotypes to best suit difficult environments (Allen, 1877). Jordan (1891) stated that fish from colder waters (higher latitudes, open ocean, deep waters) tended to have more vertebrae than related species from warmer waters (lower latitudes, coastal seas, shallow waters). Jordan

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suspected that temperature shaped the biotic and abiotic interactions that formed the selective environment of the species. Through a process Jordan described as ‘ichthyization’, warm-water species evolved reduced vertebral number as a consequence of skeletal specialization driven by enhanced competition (Jordan, 1891). He later reversed the order of events when fossil evidence revealed higher vertebral number to be the derived phenotype; relaxed selection under colder temperatures reduced skeletal specialization (Jordan, 1905). Although Jordan’s explanation of his rule is largely dismissed today [but see McDowall (2008)], Jordan’s rule continues to be a focus of research. In many (Billerbeck et al., 1997; McDowall, 2003a; McBride & Horodysky, 2004; Yamahira et al., 2006; Barriga et al., 2013), but not all (Resh et al., 1976; Shikano & Merilä, 2011) investigated species, vertebral number has been shown to increase linearly with increasing latitude. In some cases, temperature has been explicitly associated with vertebral number (Tåning, 1952; Lindsey, 1954; Seymour, 1959; Lindsey & Harrington, 1972; Baumann et al., 2012; Reimchen & Cox, 2015; Ackerly & Ward, 2016).

Changes to vertebral number have functional consequences for flexibility (Long & Nipper, 1996; McDowall, 2003a), maximum body curvature for escape swimming (Brainerd & Patek, 1998), body elongation associated with ambush predation (Maxwell & Wilson, 2013), burst swimming (Swain, 1992a) and C-start velocity (Ackerly & Ward, 2016). Although the reasons are poorly understood, vertebral number is an important differentiator of fish stocks (Swain et al., 2001), ecomorphs (Aguirre et al., 2014) and life history strategies (McDowall, 2003b), affects female reproductive investment in Esox lucius L. 1758 (Tibblin et al., 2016) and is under selective predation in multiple fish species (Swain, 1988; Ackerly & Ward, 2016; Tibblin et al., 2016). Jordan’s rule may persist in part as a means for larvae to cope with the challenges of swimming in colder or more viscous waters (Fuiman & Batty, 1997; Hunt von Herbing, 2002). Despite a wealth of effort made to detect Jordan’s rule, in most systems it is not known whether temperature-induced plasticity, local adaptation, or both are contributing to these patterns (Yamahira & Nishida, 2009; Baumann et al., 2012). Furthermore, it has been argued that Jordan’s rule is superfluous and that Bergmann’s rule (Bergmann, 1847), in which body size increases with decreasing temperature, and pleomerism (Lindsey, 1975), in which larger individuals have more vertebrae, are together sufficient to explain Jordan’s rule.

The marine three-spined stickleback Gasterosteus aculeatus L. 1758 is an ideal system in which to study Jordan’s rule. Some causes of variation in vertebral number within G. aculeatus populations have been reported, but to date no large-scale study on Jordan’s rule exists in this species. Vertebral number is moderately heritable \( h^2_N = 0.37–0.55; \) Hermida et al., 2002; Alho et al., 2011) with known quantitative trait loci (QTL; Berner et al., 2014; Miller et al., 2014). Genetic differentiation has been documented among some anadromous or marine G. aculeatus (Catchen et al., 2013; DeFaveri et al., 2013), suggesting that selection could shape Jordan’s rule in the marine environment. Although the range of temperatures that G. aculeatus experience in the wild have not been measured, several studies have shown the adaptive significance of vertebral number in this species (Swain & Lindsey, 1984; Swain, 1992b; Walker & Bell, 2000; Spoljaric & Reimchen, 2007; Aguirre et al., 2014), including the ratio of caudal to abdominal vertebrae (Swain, 1992a; Aguirre et al., 2014). Juvenile G. aculeatus with more vertebrae prefer cooler waters, potentially to optimize swimming efficiency under a given temperature–viscosity (Reimchen & Cox, 2015). Given that juveniles may migrate >100 km offshore to escape predation

greater flexibility and predator-escape abilities would seem important in colder, more viscous waters.

Temperature-induced plasticity also influences vertebral number. In G. aculeatus, vertebral number is established prior to hatching (Lindsey, 1962). Vertebral number is plastically influenced by temperature during this brief developmental window, with intermediate developmental temperatures of 18–24°C producing the lowest vertebral counts in a number of G. aculeatus genotypes (Lindsey, 1962). Assuming that latitudinal variation is associated with variation in developmental temperature, plasticity alone could account for Jordan’s rule. The role of plasticity can be supported or discounted by observing how other temperature-induced meristic traits vary with latitude. Lindsey (1962) found that numbers of dorsal- and anal-fin rays and basals increased with decreasing temperature, while pectoral-fin rays exhibited an inverted-V reaction norm. Fin rays and vertebral number have different levels of heritability in G. aculeatus (Hermida et al., 2002), with different QTLs (Miller et al., 2014) and are not correlated with one another in the wild in related Apeltes quadracus (Mitchill 1815) (Krueger, 1961). Therefore, observing patterns of association between latitude and other meristic traits can help disentangle the relationship between Jordan’s rule, local adaptation and plasticity.

Here, Jordan’s rule is tested by investigating variation in vertebral number for marine G. aculeatus found along a 21.8° latitudinal cline. The following questions were considered: does the number of vertebrae increase with increasing latitude in G. aculeatus; do Bergmann’s rule and pleomerism explain Jordan’s rule, in effect rendering Jordan’s rule superfluous; is Jordan’s rule found in both sexes and if so are there differences between the sexes that could suggest a role for selection; are other meristic traits that are known to be plastically affected by developmental temperature also correlated with latitude? Collectively, these questions seek to test the applicability of Jordan’s rule to G. aculeatus and provide a first step in determining its causes and consequences.

MATERIALS AND METHODS

COLLECTION AND SAMPLING SITES

Gasterosteus aculeatus were collected with minnow traps or kick seines during August 2012 in Alaska and July 2013 at all other localities. Sampling locations extended along 21.8° latitude (Fig. 1): Elkhorn Slough, Monterey Bay, California (CA01: 36° 49’ 45” N; 121° 44’ 07” W, n = 35), Doran Park, Bodega Bay, California (CA02: 38° 18’ 52” N; 123° 01’ 55” W, n = 48), Arcata Marsh, Arcata, California (CA03: 40° 51’ 23 N, 124° 05’ 24” W, n = 46), South Slough, Coos Bay, Oregon (OR01: 43° 17’ 35” N; 124° 19’ 26” W, n = 19), Tillamook Bay, Oregon (OR02: 45° 28’ 52” N; 123° 53’ 49” W, n = 50), Bamfield Inlet, Vancouver Island, British Columbia (BC01: 48° 49’ 55” N; 125° 08’ 17” W, n = 51) and Swikshak Lagoon, Alaska (AK01: 58° 37’ 14” N; 153° 44’ 44” W, n = 31). Most sampling locations were sheltered saltwater marshes with a muddy benthos. Males were often found in full breeding colour. Salinity varied at low tide from 12 (OR02) to over 25 (all other sites) ppt. All G. aculeatus were ≥30 mm standard length (L₅). Gasterosteus aculeatus were euthanized using buffered MS-222 or eugenol and preserved in 70% ethanol. Fin clips were preserved in 95% ethanol for later sex determination. All collections were conducted in accordance with Canadian Council on Animal Care guidelines (AUP AC13–0040) and state–provincial–national collection and import permits.
SEX DETERMINATION

DNA was extracted from fin clips using the phenol–chloroform method (Chomczynski & Sacchi, 1987). Sex was determined using primers developed by Peichel et al. (2004) that amplify sex-specific alleles at the idh locus. Alleles were visualized in a 2% agarose gel.

SAMPLE PREPARATION AND MEASUREMENTS

Radiographs were taken of individual fish positioned right laterally using a cabinet X-ray apparatus (Kubtec–XPERT 80-L www.kubtec.com), at 35 kV and 1000 μA for 15 s with lateral projection. Total, caudal and abdominal vertebrae were counted from the digital image using ImageJ (www.imagej.nih.gov), beginning with the basioccipital and ending with the vertebra preceding the urostyle (Fig. 2). Caudal vertebrae were assigned as per Aguirre et al. (2014), with the first caudal vertebra usually readily distinguished by the presence of a haemal spine and haemal arch, a lack of ribs and a close association with the first anal pterygiophore. Transitional vertebrae that had haemal arches but no haemal spines, or had qualitatively short haemal arches that were not associated with the first anal pterygiophore, were scored as abdominal vertebrae, following Aguirre et al. (2014). Standard length (mm) was measured in ImageJ.

Fish were stained in alizarin red for visualization of fin rays and basals. Dorsal-fin rays were counted beginning at the first soft ray posterior to the third dorsal spine. Anal-fin ray counts included the first spiny fin ray. Pectoral-fin ray counts were calculated as the average from both pectoral fins, as asymmetry was sometimes present. Branched rays were counted as single fin rays. Dorsal and anal-fin basals were also counted, but as each fin ray always had a basal associated with it, only counts of basals posterior to the last associated fin ray were included. These could be thought of as extra basals that extend beyond the fin. Three BC01 fish were sent to
another facility before these additional phenotypes could be measured, so for all fin-ray and basal counts the sample size was reduced to 48 for this population.

STATISTICS

The $\chi^2$-tests were used to test for equality in sex ratio within each site. Logistic regression was used to determine the association between sex ratio and latitude. Since ecogeographic rules usually assume a linear relationship with latitude, linear regression was used as a first approximation of the influence of latitude on vertebral number (total, abdominal and caudal) and $L_S$. Analysis of variance (ANOVA) was conducted for each phenotype using site as a factor, with post-hoc tests used to contrast all sampling sites. It became apparent that sites south of British Columbia showed little variation, so vertebral number and $L_S$ were again tested using a linear regression for only these southern sites. Since Jordan’s rule and Bergmann’s rule make different but potentially closely related predictions, a linear regression was run for Jordan’s rule with $L_S$ as a covariate. The interaction term and main effects were systematically dropped and the model with the lowest Akaike Information Criterion (AIC) selected for further analysis. Type II sums of squares were calculated for the final model.

Welch’s $t$-test was used to assess differences between the sexes for vertebral number (total, abdominal, caudal) and $L_S$. Linear regressions for Jordan’s rule and Bergmann’s rule were reanalysed separately for each sex. Spearman correlations for $L_S$ v. vertebral number were also reanalysed separately for each sex.

Spearman correlations were calculated for comparisons between vertebral number (total, abdominal, caudal), $L_S$ and average pectoral-fin ray, dorsal-fin ray, anal-fin ray, dorsal-fin basal and anal-fin basal number, with $\alpha$ corrected to 0.001. Linear regressions were conducted for all fin rays and basals in association with latitude. Pleomerism was tested using a Pearson correlation between the average vertebral number for a population and the maximum $L_S$ sampled from the population (Lindsey, 1975). All statistical analyses were conducted in R 3.2.1 (www.r-project.org).

RESULTS

SEX

The proportion of males and females differed among sites (Table I). Three sites (CA02, CA03 and OR01) had male:female ratios that did not differ from 1:1. Of the rest, CA01 had a M:F ratio of 3:1, OR02 and AK01 of 1:3 and BC01 of 12:1 ($\chi^2 > 6.63$ for all four tests, d.f. 1, $P < 0.01$). These site-specific differences were not correlated with latitude (logistic regression, $z = -1.5$, d.f. 279, $P > 0.1$).
Table I. Mean ± s.d. phenotypes of *Gasterosteus aculeatus* collected along the Pacific coast of North America from central California to Alaska.

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Sampling date</th>
<th>Sample size (n)</th>
<th>Sex (M:F)</th>
<th>$L_S$ (mm)</th>
<th>$L_{S_{\text{max}}}$ (mm)</th>
<th>Total vertebrae $(n)$</th>
<th>Abdominal vertebrae $(n)$</th>
<th>Caudal vertebrae $(n)$</th>
<th>Dorsal-fin ray $(n)$</th>
<th>Anal-fin ray $(n)$</th>
<th>Pectoral fin-ray $(n)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA01</td>
<td>36° 49'</td>
<td>July 2013</td>
<td>35</td>
<td>26.9*</td>
<td>42.3 ± 3.6$^{AB}$</td>
<td>50.22</td>
<td>31.6 ± 0.8$^{A}$</td>
<td>15.8 ± 0.7$^{AC}$</td>
<td>15.8 ± 0.7$^{A}$</td>
<td>11.3 ± 0.8$^{AB}$</td>
<td>9.2 ± 0.7$^{A}$</td>
<td>10.0 ± 0.2$^{B}$</td>
</tr>
<tr>
<td>CA02</td>
<td>38° 18'</td>
<td>28</td>
<td>48</td>
<td>20:28</td>
<td>43.2 ± 3.4$^{B}$</td>
<td>50.90</td>
<td>31.6 ± 0.6$^{A}$</td>
<td>15.8 ± 0.6$^{AC}$</td>
<td>15.8 ± 0.8$^{A}$</td>
<td>11.3 ± 0.9$^{AB}$</td>
<td>8.8 ± 0.9$^{A}$</td>
<td>10.3 ± 0.4$^{A}$</td>
</tr>
<tr>
<td>CA03</td>
<td>40° 51'</td>
<td>22, 29</td>
<td>46</td>
<td>28:18</td>
<td>39.2 ± 5.8$^{A}$</td>
<td>54.16</td>
<td>31.5 ± 0.6$^{A}$</td>
<td>15.5 ± 0.5$^{A}$</td>
<td>16.0 ± 0.6$^{A}$</td>
<td>11.3 ± 0.7$^{AB}$</td>
<td>9.1 ± 0.6$^{A}$</td>
<td>10.0 ± 0.2$^{B}$</td>
</tr>
<tr>
<td>OR01</td>
<td>43° 17'</td>
<td>19</td>
<td>7:12</td>
<td>41.9 ± 7.0$^{AB}$</td>
<td>52.15</td>
<td>31.3 ± 0.6$^{A}$</td>
<td>15.8 ± 0.5$^{AC}$</td>
<td>15.5 ± 0.7$^{A}$</td>
<td>15.9 ± 0.6$^{A}$</td>
<td>10.7 ± 0.8$^{A}$</td>
<td>9.0 ± 0.6$^{A}$</td>
<td>9.9 ± 0.2$^{B}$</td>
</tr>
<tr>
<td>OR02</td>
<td>45° 28'</td>
<td>14</td>
<td>50</td>
<td>13:37*</td>
<td>47.3 ± 3.6$^{D}$</td>
<td>54.72</td>
<td>31.4 ± 0.7$^{A}$</td>
<td>15.5 ± 0.7$^{A}$</td>
<td>16.8 ± 0.6$^{A}$</td>
<td>12.3 ± 0.7$^{C}$</td>
<td>10.5 ± 0.6$^{B}$</td>
<td>10.0 ± 0.1$^{B}$</td>
</tr>
<tr>
<td>BC01</td>
<td>48° 49'</td>
<td>7</td>
<td>51*</td>
<td>47.4*</td>
<td>52.0 ± 2.4$^{E}$</td>
<td>57.23</td>
<td>32.3 ± 0.7$^{B}$</td>
<td>15.5 ± 0.5$^{A}$</td>
<td>16.8 ± 0.7$^{B}$</td>
<td>12.3 ± 0.7$^{C}$</td>
<td>10.5 ± 0.6$^{B}$</td>
<td>10.0 ± 0.1$^{B}$</td>
</tr>
<tr>
<td>AK01</td>
<td>58° 37'</td>
<td>August 2012</td>
<td>31</td>
<td>8:23*</td>
<td>58.8 ± 8.1$^{F}$</td>
<td>75.05</td>
<td>32.3 ± 0.7$^{B}$</td>
<td>16.2 ± 0.4$^{BC}$</td>
<td>16.1 ± 0.5$^{A}$</td>
<td>11.6 ± 0.9$^{B}$</td>
<td>9.3 ± 0.7$^{A}$</td>
<td>10.0 ± 0.2$^{B}$</td>
</tr>
</tbody>
</table>

$L_S$, Standard length; CA, California; OR, Oregon; BC, British Columbia; AK, Alaska. Different superscript letters denote significantly different ANOVA post-hoc Tukey HSD contrasts at $\alpha < 0.0023$. *, significantly different ($P < 0.05$) from expectation of 1:1.

#sample includes 48 for dorsal, anal, and pectoral-fin phenotypes.
Table II. The frequency distribution of *Gasterosteus aculeatus* exhibiting different numbers of total, abdominal, and caudal vertebrae within populations along the Pacific coast of North America from central California to Alaska (N.B. no individual had 18 abdominal vertebrae)

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude N</th>
<th>Total</th>
<th>Abdominal</th>
<th>Caudal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>CA01</td>
<td>36° 49'</td>
<td>2</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>CA02</td>
<td>38° 18'</td>
<td>1</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>CA03</td>
<td>40° 51'</td>
<td>2</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>OR01</td>
<td>43° 17'</td>
<td>1</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>OR02</td>
<td>45° 28'</td>
<td>3</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>BC01</td>
<td>48° 49'</td>
<td>1</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>AK01</td>
<td>58° 37'</td>
<td>0</td>
<td>3</td>
<td>18</td>
</tr>
</tbody>
</table>

CA, California; OR, Oregon; BC, British Columbia; AK, Alaska.

JORDAN’S RULE AND VERTEBRAL NUMBER

Total vertebral number varied from 30 to 34 (Table II). There was a significant relationship between vertebral number and latitude (Jordan’s rule) (Fig. 3), with vertebral number increasing 0.04 vertebrae per increase in °N (linear regression, $F_{1,278} = 35.2$, $P < 0.001$, $R^2 = 0.11$). A linear regression using only Californian and Oregon populations, however, was not significant and in the opposite direction of expectation (linear regression, $F_{1,196} = 3.4$, $P > 0.05$, $R^2 = 0.02$). From an ANOVA and post-hoc Tukey HSD test, it was apparent that vertebral counts did not vary from CA01 to OR01, nor did they vary between BC01 and AK01, but BC01 and AK01 fish had significantly more vertebrae when contrasted with fish from any other locality (Fig. 3 and Table I). The significant regression supporting Jordan’s rule across seven populations was driven by the two northernmost populations having high vertebral counts (mean ± s.d. = 32.27 ± 0.68 vertebrae) and all populations south of BC01 having consistently lower vertebral counts (mean ± s.d. = 31.51 ± 0.65).

Abdominal vertebral number varied from 14 to 19 (Table II). AK01 had significantly more abdominal vertebrae than BC01 (ANOVA with Tukey HSD $P < 0.001$) and some southern localities (Table I). Caudal vertebral number varied from 14 to 18 (Table II). There was a shift to a higher average caudal vertebral number in BC01 compared with all other sites, including AK01 (Tukey HSD $P < 0.001$) (Table I). Only caudal number varied significantly and positively with latitude (caudal: linear regression, $F_{1,278} = 18.7$, $P < 0.001$, $R^2 = 0.06$; abdominal: $P > 0.1$) (Fig. 3). The ratio of caudal to abdominal vertebrae similarly increased with latitude (linear regression, $F_{1,275} = 4.5$, $P < 0.05$, $R^2 = 0.01$). Caudal and abdominal vertebrae were negatively correlated (Spearman correlation, $S = 5.329900$, $\rho = -0.46$, $P < 0.001$) (Fig. 4).

BERGMANN’S RULE AND STANDARD LENGTH

Standard length varied among sites (Table I). The northernmost site (AK01) had the longest fish (mean $L_S$ 58.8 mm). There was a significant association between latitude and $L_S$ (Bergmann’s rule; Fig. 3), with a 0.9 mm increase in $L_S$ per °N (linear regression, $F_{1,278} = 310.2$, $P < 0.001$, $R^2 = 0.53$). Unlike Jordan’s rule, Bergmann’s rule was still supported after removing the two northernmost populations (linear regression,
Fig. 3. Mean ± s.e. of phenotypic variation in male (▲) and female (●) *Gasterosteus aculeatus* for each sampling site (Fig. 1 and Table I) along the Pacific coast of North America from central California to Alaska (NB only four females were captured at 48.8° N). (a) Standard length ($L_S$), (b) total number of vertebrae (Vert.), (c) number of abdominal vertebrae (Abdom.) and (d) number of caudal vertebrae (Caudal).
The sexes differed for a number of traits (Figs 3–6). Males had significantly more vertebrae overall than females (31·87 v. 31·57, Welch’s $t = -3·4$, d.f. = 271, $P < 0·001$) despite having smaller $L_S$ on average (45·4 mm v. 47·5 mm, Welch’s $t = 2·3$, d.f. = 262, $P < 0·05$). Jordan’s rule still applied, however, for both sexes (linear
Fig. 5. Relationship between mean ± s.e. standard length (L\textsubscript{S}) and mean ± s.e. total vertebral number, separated by population and sex (\(r^2 = 0.34, P < 0.001\)). □ or ▧, female; □ or □, male; △, CA01, Elkhorn Slough, Monterey Bay, California; □, CA02, Doran Park, Bodega Bay, California; ◊, CA03, Arcata Marsh, Arcata, California; ○, OR01, South Slough, Coos Bay, Oregon; X, OR02, Tillamook Bay, Oregon; ■, BC01, Bamfield Inlet, Vancouver Island, British Columbia; □ and △, AK01, Swikshak Lagoon, Alaska (NB only four females were captured from BC01).

regression, males: \(F_{1,147} = 23.0, P < 0.0001, R^2 = 0.14\); females: \(F_{1,129} = 18.3, P < 0.001, R^2 = 0.12\). Females had more abdominal vertebrae than males (15.9 v. 15.5, Welsh’s \(t = 4.3, d.f. = 254, P < 0.001\)). Males had more caudal vertebrae than females (16.3 v. 15.7, Welsh’s \(t = -7.2, d.f. = 278, P < 0.001\)). Jordan’s rule, however, was caused by an increase in the ratio of caudal to abdominal vertebrae in males only (linear regression, males: \(F_{1,147} = 8.8, P < 0.01, R^2 = 0.05\); females: \(F_{1,126} = 1.6, P > 0.05\). Bergmann’s rule applied for both sexes (linear regression, males: \(F_{1,147} = 174.6, P < 0.001, R^2 = 0.54\); females: \(F_{1,129} = 133.9, P < 0.001, R^2 = 0.51\). Similarly, the \(L\textsubscript{S}–\)vertebral number relationship held for both sexes (Spearman correlation, males: \(S = 312170, \rho = 0.43, P < 0.001\); females: \(S = 3121704, \rho = 0.27, P < 0.01\).

FIN RAYS AND BASALS

Variation in all meristics showed some association with latitude (Fig. 6). Pectoral-fin ray counts decreased by 0.01 rays per increase in °N (linear regression, \(F_{1,275} = 13.6, P < 0.001, R^2 = 0.05\)). Other traits increased with latitude: dorsal-fin ray counts (linear regression, \(F_{1,275} = 12.1, P < 0.001, R^2 = 0.04\)), dorsal-fin basal counts (linear regression, \(F_{1,275} = 5.0, P < 0.05, R^2 = 0.02\)), anal-fin ray counts (linear regression, \(F_{1,275} = 18.7, P < 0.001, R^2 = 0.06\)) and anal-fin basal counts (linear regression, \(F_{1,275} = 12.2, P < 0.001, R^2 = 0.04\)).

Dorsal and anal-fin rays were positively correlated with each other and with \(L\textsubscript{S}\), total vertebral number and caudal vertebral number. Anal and dorsal-fin basals were also positively correlated with each other and were negatively correlated with
their respective fin rays. Pectoral-fin rays showed no correlations with any trait (Table III).

**DISCUSSION**

**JORDAN’S RULE**

Phenotypic variation, whether genetically or plastically based, may have functional significance (Higham et al., 2016). In this study of the three-spined stickleback *G. aculeatus* vertebral number increased with increasing latitude, which is predicted by ecogeographic rules. In particular, *G. aculeatus* north of Washington had more vertebrae than their Oregonian and Californian counterparts. Miller & Hubbs (1969)
Table III. Spearman correlations for pairs of *Gasterosteus aculeatus* phenotypes along the Pacific coast of North America from central California to Alaska

<table>
<thead>
<tr>
<th></th>
<th>Total vertebrae (n)</th>
<th>$L_S$</th>
<th>Caudal</th>
<th>Abdom.</th>
<th>Pectoral</th>
<th>Dorsal</th>
<th>Anal</th>
<th>D-basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_S$</td>
<td>0.63***</td>
<td>0.33***</td>
<td>0.29***</td>
<td></td>
<td>0.01</td>
<td>0.42***</td>
<td>0.32***</td>
<td>0.41***</td>
</tr>
<tr>
<td>Caudal</td>
<td></td>
<td>0.33***</td>
<td>0.03</td>
<td>0.33***</td>
<td>0.01</td>
<td>0.27***</td>
<td>0.48***</td>
<td>0.41***</td>
</tr>
<tr>
<td>Abdom.</td>
<td>0.33***</td>
<td></td>
<td>0.03</td>
<td>0.33***</td>
<td>0.01</td>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
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<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.09</td>
<td>-0.46***</td>
<td>0.62***</td>
<td>0.06</td>
</tr>
<tr>
<td>Dorsal</td>
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<td></td>
<td>0.42***</td>
<td>-0.02</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.43***</td>
<td>-0.24***</td>
</tr>
<tr>
<td>Anal</td>
<td>0.41***</td>
<td></td>
<td>0.48***</td>
<td>0.00</td>
<td>0.00</td>
<td>0.15</td>
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<tr>
<td>D-basal</td>
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<td></td>
<td>-0.06</td>
<td>0.00</td>
<td>0.00</td>
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<td></td>
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<tr>
<td>A-basal</td>
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<td>0.04</td>
<td>0.05</td>
<td>0.01</td>
<td></td>
<td></td>
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</tbody>
</table>

$L_S$, standard length; Caudal, number of caudal vertebrae; Abdom., number of abdominal vertebrae; Dorsal, number of dorsal-fin rays; Anal, number of anal-fin rays; D-basal, number of extra dorsal-fin basals; A-basal, number of extra anal-fin basals. *** $P < 0.001$.

discovered a similar pattern in this region for freshwater *G. aculeatus*. Although the authors did not provide averages for the total population, the range of averages for different plate morphs suggested that *G. aculeatus* from Sitkalidak Island, AK (31.87 to 32.00 vertebrae) had more vertebrae than those from Carmel River, CA (31.24 to 31.33 vertebrae). The results from this study are in contrast to a study of *G. aculeatus* on the east coast, which reported no evidence for Jordan’s rule (Garside & Hamor, 1973). The latitudinal spread from that study, however, was much smaller (<6° latitude).

The amount of variation explained by latitude was low, as vertebral number did not increase from southern California to northern Oregon. Instead, vertebral number was relatively constant and low until British Columbia, with BC01 and AK01 having significantly more vertebrae. Thus although Jordan’s rule was confirmed in this system, vertebral number variation exhibited more of an abrupt transition than a smooth linear trend. More sites will have to be sampled in the future to determine where this transition begins and if it holds throughout central and northern British Columbia and western Alaska.

**Bergmann’s Rule and Pleomerism**

Latitude was more strongly associated with $L_S$ than with vertebral number, confirming Bergmann’s rule in this system (for complications and comparisons with other fish species, see Blackburn et al., 1999; Belk & Houston, 2002; Rypel, 2014). Furthermore, fish with longer $L_S$ tended to have more vertebrae, with weak evidence, despite low power, for Lindsey’s pleomerism. It has been suggested that Bergmann’s rule combined with the $L_S$–vertebral number relationship makes Jordan’s rule redundant (McDowall, 2008). That is, Jordan’s rule is simply a function of larger size being selected in colder waters. This does not fully explain results here, as latitude had a significant effect on vertebral number even when accounting for $L_S$. Furthermore, it has been proposed that Bergmann’s rule is due to increased longevity caused by slow growth and reduced competition (Angilletta & Dunham, 2003; Rypel, 2014); if so, this cannot explain Jordan’s
JORDAN’S RULE IN GASTEROSTEUS ACULEATUS

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rule in *G. aculeatus* as vertebral number is determined early in development (Lindsey, 1962). Given that marine *G. aculeatus*, including juveniles, migrate vast distances (Cowen et al., 1991), both Bergmann’s rule and Jordan’s rule could be adaptive, particularly for the viscous waters of the north (Hunt von Herbing, 2002; McDowall, 2003a; Aguirre et al., 2014).

SEXUAL DIMORPHISM

Jordan’s rule was confirmed in both sexes, but the underlying patterns differed. Male *G. aculeatus* had more caudal vertebrae than females, while females had more abdominal vertebrae than males. There was a tendency for individuals with more caudal vertebrae to have fewer abdominal vertebrae and *vice versa*, patterns that have also been reported for Alaskan freshwater and anadromous *G. aculeatus* (Aguirre et al., 2014). The gain in abdominal vertebrae in females, however, did not fully compensate for the gain in caudal vertebrae in males, such that males had more total vertebrae than females. This is consistent with observations on freshwater *G. aculeatus* from the Haida Gwaii, BC (Reimchen & Nelson, 1987). Males and females both showed increased vertebral number with latitude, but only males showed an increase in the ratio of caudal to abdominal vertebrae. In other words, Jordan’s rule occurred due to an increase in caudal vertebral number with increasing latitude in males, but an increase in total vertebral number in females. Although the functional significance of altering these ratios is not completely understood, a higher ratio is important for predator avoidance (Swain, 1992a), while a lower ratio may be necessary for the expansion of the abdominal cavity associated with egg production (Aguirre et al., 2014). These results suggest a potential role for selection in shaping Jordan’s rule in this species.

PLASTICITY AND MERISTIC TRAITS

Lindsey (1962) explored temperature-induced plasticity in a variety of meristic phenotypes in a freshwater population of *G. aculeatus*. He found that total vertebral number was plastically influenced by temperature according to a V-shaped reaction norm, with the trough of the V located between 18 and 24° C, depending on the genotype (in this case, fish with 2–5 plates). Decreasing the hatching temperature below this peak resulted in increasing numbers of vertebrae, although temperatures below 16° C were not examined. Dorsal and anal-fin rays and, to a lesser extent, dorsal and anal-fin basals, also increased with decreasing temperature, while pectoral-fin rays showed an inverted V relationship with temperature (Lindsey, 1962). Since the breeding grounds of *G. aculeatus* in central California can experience temperatures as high as the temperatures that induced the fewest vertebrae in Lindsey’s experiment (Fig. S1, Supporting Information), similar patterns to Lindsey’s observations can be anticipated if developmental temperature is driving Jordan’s rule. These predictions did in fact hold true, although the amount of variation explained by latitude for each meristic trait was always <10%.

More information is needed to elucidate the mechanisms underlying the correlations between meristic traits and latitude. Little is known about genetic covariation between different meristic traits in fish, nor is much known about the functional significance of such covariation [see Chadwell & Ashley-Ross (2012) for a discussion on the functional significance of fins in general]. It is interesting that dorsal and anal-fin ray numbers were strongly correlated. In *G. aculeatus* and many other fish species

the torques produced by dorsal and anal fins balance each other out during sustained swimming (Standen & Lauder, 2005).

CAUSES OF JORDAN’S RULE

At least three different definitions of Jordan’s rule have been used over the years. Jordan’s initial formulation of his rule was general and lacked a firm mechanism. It was applied only to related species or genera, with selection on heritable genetic variation as the cause (Günther, 1862; Jordan, 1891). The second version of Jordan’s rule was developed by Hubbs (1922) with a focus on within-population variation of a single species. For Hubbs, Jordan’s rule was a direct consequence of temperature affecting the rate of early development (McDowall, 2008). Between these two extremes, the current definition measures interpopulational variation within a single species spread over a broad latitudinal range (McDowall, 2003; Baumann et al., 2012; Barriga et al., 2013). Latitude is considered a proxy for temperature, with an assumed linear relationship. Under this definition there is no a priori cause (selection or development) for Jordan’s rule.

The proposed abiotic factors that influence Jordan’s rule include light intensity (MacCrimmon & Kwain, 1969), oxygen concentration (Garside, 1966), salinity (Fahy & O’Hara, 1977) and temperature. Overall, temperature has the greatest empirical support (Jordan, 1891; Hubbs, 1922; Baumann et al., 2012; Ackerly & Ward, 2016). An examination of Bergmann’s rule in multiple fish species reported similar results irrespective of whether body size was regressed on latitude or temperature (Rypel, 2014). Vertebral number, unlike body size, is set early in development, so it is unclear the extent to which these rules are comparable.

Jordan’s rule could be caused by plasticity, selection on genetic variation, or a combination of the two. The best experimental evidence for the causes of Jordan’s rule comes from silversides (Baumann et al., 2012). Atlantic Menidia menidia (L. 1766) exhibit a stronger regression of vertebral number on latitude than Atherinops affinis (Ayres 1860) from the Pacific Ocean, presumably associated with the stronger latitudinal temperature gradient in the Atlantic Ocean. Plasticity only partially explained this variation in a common-garden environment, suggesting Jordan’s rule was caused in part by natural selection on genetically-heritable variation.

Marine G. aculeatus are generally considered to be genetically homogeneous along the Pacific coast (Withler & McPhail, 1985; Hohenlohe et al., 2010), suggesting plasticity may explain Jordan’s rule. It is possible however that some degree of population structure exists in the marine environment (Catchen et al., 2013; DeFaveri et al., 2013), so genetic causes for Jordan’s rule cannot be ruled out. The results here are consistent with a dual role of selection and plasticity; variation in meristic traits suggests that populations with more vertebrae developed under cooler temperatures, while sex-specific differences in Jordan’s rule suggest that selection has shaped the responses each sex has to temperature. Further investigation is clearly required to disentangle these complex relationships.

The linear relationship between vertebral number and latitude, although significant, did not account for much of the total variation in vertebral number. This is in part because populations south of BC01 had similar low numbers of vertebrae, while BC01 and AK01 had similar high numbers of vertebrae. Plasticity and selection could explain these trends. Plasticity because, although average sea surface temperatures
JORDAN’S RULE IN GASTEROSTEUS ACULEATUS

vary linearly with latitude (Baumann et al., 2012), marine G. aculeatus may seek similar preferred temperatures along the coast in which to build their nests (Mori, 1994) as a means of optimizing the amount of oxygen they need to provide to their eggs (Reebs et al., 1984; Hopkins et al., 2011). Selection because waters at a range of warmer temperatures vary less in viscosity than do waters at a similar range of cold temperatures (Hunt von Herbing, 2002), such that predator escape velocity would not be affected across a range of warm temperatures.

FUTURE DIRECTIONS

Gasterosteus aculeatus is an important model within both evolutionary and developmental biology. In this study we have shown that Jordan’s rule exists in marine G. aculeatus, but a number of questions have been highlighted that require further exploration. What is the functional significance of Jordan’s rule in G. aculeatus? What is the influence of temperature and what temperatures do G. aculeatus experience during their life history?

Ecogeographic rules were once used to provide compelling evidence for the role of natural selection, but today the causes of these rules are harder to disentangle. Although selection is certainly responsible for some rules or some instances of rules (Allen’s rule; Allen, 1877), plasticity alone may explain other patterns. Individuals along an entire geographic range could have the capacity to produce any of the phenotypes found along the range. Given that many species lose the capacity for plasticity when plasticity is no longer expressed (Pigliucci et al., 2006), one must ask why plasticity would be maintained in the context of ecogeographic rules. Even if selection is not the direct cause of the developed phenotype, understanding its role may still hold the key to explaining the capacity for phenotypic change. Studying the ecological and evolutionary processes underlying ecogeographic patterns will increase our understanding of the processes generating predictable diversity that led to these rules in nature.

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Supporting Information

Supporting Information may be found in the online version of this paper:
Fig. S1. Mean ± s.d. monthly (J, January to D, December) local seawater temperatures 2010–2014 of specific bays along the Pacific coast of North America: Elkhorn Slough, California (—–), Coos Bay, Oregon (…..), and Padilla Bay, Washington (— – -). Adapted from NOAA National Estuarine Research Reserve System (https://coast.noaa.gov/nerrs/).
Appendix S1. Temperature at the breeding grounds.
Appendix S2. Phenotypic information per fish, including sex, vertebral number (total, abdominal, and caudal), standard length, average number of pectoral-fin rays, and number of anal-fin rays and basals, and dorsal-fin rays and basals.

References


