RESEARCH ARTICLE

Genetically and environmentally mediated divergence in lateral line morphology in the Trinidadian guppy (*Poecilia reticulata*)

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SUMMARY

Fish and other aquatic vertebrates use their mechanosensory lateral line to detect objects and motion in their immediate environment. Differences in lateral line morphology have been extensively characterized among species; however, intraspecific variation remains largely unexplored. In addition, little is known about how environmental factors modify development of lateral line morphology. Predation is one environmental factor that can act both as a selective pressure causing genetic differences between populations, and as a cue during development to induce plastic changes. Here, we test whether variation in the risk of predation within and among populations of Trinidadian guppies (*Poecilia reticulata*) influences lateral line morphology. We compared neuromast arrangement in wild-caught guppies from distinct high- and low-predation population pairs to examine patterns associated with differences in predation pressure. To distinguish genetic and environmental influences, we compared neuromast arrangement in guppies from different source populations reared with and without exposure to predator chemical cues. We found that the distribution of neuromasts across the body varies between populations based on both genetic and environmental factors. To the best of our knowledge, this study is the first to demonstrate variation in lateral line morphology based on environmental exposure to an ecologically relevant stimulus.

Key words: neuromast, intraspecific variation, differential selection, predator cues.

INTRODUCTION

The capacity to detect and respond to environmental stimuli is essential to an organism's ability to survive and reproduce, and therefore is likely under strong selection. As a result, organisms typically have sensory systems that are adapted to the environments in which they live. Because intraspecific variation is the raw material for adaptive differentiation among species, examining variation within a single species contributes to our understanding of species divergence and evolution. To infer the potential for adaptive evolution from patterns of intraspecific variation, we must distinguish the variation due to underlying genetic differences between individuals from the variation induced by the environment. Here, we examine genetic and environmental influences on lateral line morphology.

The lateral line is a mechanosensory system present in aquatic vertebrates, including all fishes, larval amphibians and aquatic adult amphibians (e.g. Webb, 1989a; Coombs et al., 1992). The sensory end organs of the lateral line are the neuromasts, which are derived from epithelial placodes on the head and function in the detection of water movements (e.g. Dijkgraaf, 1963; Bleckmann, 1993). A neuromast is composed of a collection of hair cells. The cell bodies of the hair cells are surrounded by support cells and the ciliary bundle on the apical surface of each hair cell is embedded in a gelatinous cupula (Webb, 1989a; Coombs et al., 1992). Water displacements shear the cupula parallel to its base, bending the stereocilia and triggering responses in the nerves innervating the hair cells (e.g. Kroese and Van Netten, 1989; Coombs and Van Netten, 2005). Hair cells transform the complex mechanosensory information from the

fluid environment into neural signals, and the central nervous system integrates information from all over the body to create a hydrodynamic image of the environment (Engelmann et al., 2000; Montgomery et al., 2003; Coombs and Vannetten, 2005; Bleckmann, 2008). In fishes, two types of neuromasts exist: superficial neuromasts, which are found on the surface of the skin, and canal neuromasts, which are located in ossified canals or open grooves that are partially embedded in dermal bones of the head or scales of the trunk. Superficial neuromasts project directly into the external environment, whereas canal neuromasts lie in canals connected to the external environment by intermittently placed pores (Webb, 1989a; Webb, 1989b; Webb and Shirey, 2003). Superficial and canal neuromasts respond to different frequencies; superficial neuromasts act as low-pass filters tuned to low frequency motion, while canal neuromasts detect high frequency motion (e.g. Bleckmann, 1993; Coombs and Braun, 2003). Owing to their differences in frequency sensitivity, it is generally accepted that superficial and canal neuromasts are functionally distinct; however, understanding the relationship between form and function across species – and the selective pressures important in driving lateral line divergence – remains challenging (Coombs et al., 1992; Montgomery et al., 1995; Bleckmann and Zelick, 2009).

Neuromasts are typically found covering the head, in particular, surrounding the eyes, and in one or more lines running laterally along the body (Northcutt, 1989; Webb, 1989a). Substantial variation exists among species in the overall arrangement of superficial neuromasts, the relative abundance of canal *versus* superficial neuromasts, and canal morphology (Webb, 1989a; Coombs et al.,

1992). Interspecific differences in these characteristics have been linked to functional specializations in some taxa (Northcutt, 1989; Webb, 1989a; Montgomery et al., 1994), but intraspecific variation has been described in only a limited number of species [*Gasterosteus aculeatus* (Wark and Peichel, 2010); *Astyanax mexicanus* (Yoshizawa et al., 2010); and *Pungitius pungitius* (Trokovic et al., 2011)].

The number and location (i.e. the distribution) of neuromasts across the body and head determine the ability of fish to detect stationary as well as moving stimuli, and lateral line sensory information thus influences many aspects of fish behaviour. For example, fish use their lateral line system to examine novel objects (Teyke, 1990; Burt de Perera, 2004), detect prey items (Hoekstra and Janssen, 1985; Janssen et al., 1999; Yoshizawa et al., 2010), monitor the movement of conspecifics (Partridge and Pitcher, 1980; Partridge et al., 1980; Satou et al., 1994; Faucher et al., 2010) and predators (McHenry et al., 2009), and maintain position in flowing water (Sutterlin and Waddy, 1975; Montgomery et al., 1997). Consequently, diverse selection pressures acting on a range of behaviours have the potential to drive lateral line evolution. We took advantage of the Trinidadian guppy (*Poecilia reticulata* Peters 1859) system to examine intraspecific patterns of lateral line divergence across predation regimes.

The Trinidadian guppy system

Trinidadian guppies are a model system in ecological and evolutionary research because they provide an opportunity to study natural selection and adaptive evolution in the wild (Houde, 1997; Magurran, 2005; Reznick et al., 2008). Much of the adaptive variation observed in guppies is associated with differences in predation pressure (Endler, 1995; Houde, 1997; Magurran, 2005; Reznick et al., 2008). In downstream localities, guppies co-occur with a diversity of piscivorous fish, most notably the pike cichlid, *Crenicichla frenata* (Endler, 1978; Endler, 1995). Guppy populations from these localities experience intense predation and are classically referred to as 'high predation' (reviewed in Magurran, 2005). Piscivorous predators are prevented from upstream migration by waterfall barriers, resulting in 'low-predation' localities in shallow, high-elevation streams. Here, guppies predominantly co-occur only with the killifish *Rivulus hartii*, a minor guppy predator that primarily preys on juveniles (Seghers, 1973). High-predation guppies have repeatedly and independently colonized and adapted to low-predation environments (Alexander and Breden, 2004; Barson et al., 2009; Willing et al., 2010), resulting in parallel changes in life history traits, morphology and behaviour (Endler, 1995; Reznick et al., 1990; Reznick et al., 1997; Magurran, 2005; Reznick et al., 2008). While many of the differences observed between high- and low-predation guppies are attributed to predation pressure, high- and low-predation localities also differ in food availability, population density, water characteristics, light levels and primary productivity, all of which may also contribute to adaptive divergence (Grether et al., 2001; Reznick et al., 2001; Zandonà et al., 2011). Guppies derived from high- and low-predation source populations show genetically based phenotypic differences (e.g. Seghers, 1974; Breden et al., 1987; Magurran and Seghers, 1991; Ghalambor et al., 2004; Huizinga et al., 2009), but many traits also change in response to environmental exposure to predator cues during development (Nordell, 1998; Dzikowski et al., 2004; Botham et al., 2006; Gosline and Rodd, 2008; Torres-Dowdall et al., 2012; Ruell et al., 2013). Thus, common garden breeding experiments that rear guppies from different populations under different environmental conditions enable us to distinguish between genetic and environmental influences on phenotype.

We predicted that lateral line morphology would differ between high- and low-predation guppies, as the expression of morphology-, anti-predator-, courtship- and swimming-related phenotypes, which differ among populations, is linked to lateral line development and function. The arrangement of neuromasts on the caudal fin of the guppy has been previously described (Wada et al., 2008); however, a description of neuromast distribution across the body does not exist in guppies, nor have differences between populations been examined. In the present study, we first described the lateral line morphology of the guppy. We next examined neuromast distribution in wild-caught fish from distinct high- and low-predation population pairs to understand whether shared environmental conditions are associated with parallel changes in independently derived population pairs. Finally, we described neuromast arrangement in fish reared in the laboratory with and without predator chemical cues in order to differentiate between genetic and environmental influences on lateral line morphology.

MATERIALS AND METHODS
Collection and rearing

We collected wild-caught individuals in March 2011 from high- and low-predation population pairs in two drainages: the Oropuche drainage (Oropuche high- and low-predation) and the Marianne drainage (Marianne high- and low-predation) under a scientific collecting permit from the Fisheries Division of the Ministry of Agriculture, Land and Marine Resources of the Republic of Trinidad and Tobago. Past genetic studies have established that low-predation populations are derived from adjacent high-predation populations downstream within each drainage, thus differences between population pairs in distinct drainages are independently derived (e.g. Alexander and Breden, 2004; Barson et al., 2009; Willing et al., 2010). Wild-caught fish were transported to our fish facility at Colorado State University (CSU) and all data collection was completed within 6 weeks of their arrival (*N*=15–22 individuals per population). All fish were sexually mature at the time of capture. All animal rearing was carried out in accordance with CSU IACUC protocol 09-1348A, and DASPEI procedures (see below) conformed with CSU IACUC protocol 10-2263A.

We used a laboratory breeding design to determine whether population differences in the wild reflect genetic and/or environmentally induced differences. Gravid wild-caught females were collected in 2008 from the downstream Guanapo high-predation (GH-HP) and the upstream Taylor low-predation (TL-LP) localities within the Guanapo river drainage and used to generate laboratory breeding stocks. We established 20–30 unique second-generation family lines from each population. First generation laboratory-born fish were separated by sex and kept in isolated tanks under identical conditions. Female and male first generation fish were uniquely crossed to generate the second generation of laboratory-born fish used in this study (*N*=20–35 per group). This breeding design maintains the genetic variation of the original wild-caught females while minimizing environmental and maternal effects, such that any differences observed between populations reared in a common laboratory environment can be attributed to genetic differences (see Reznick and Bryga, 1987). In order to test environmental influences on lateral line development, second generation siblings were split into two rearing conditions, either with (pred+) or without (pred–) predator chemical cues (see Torres-Dowdall et al., 2012; Ruell et al., 2013; Handelsman et al., 2013). In the pred– condition, fish were housed in 1.5-litre individual tanks within a re-circulating system containing only conditioned water (i.e. sterilized and carbon filtered tap water that was treated to have

a pH, hardness and chemistry similar to natural streams). In the pred+ condition, all parameters were identical, except a pike cichlid predator was housed in the sump tank of the re-circulating system and was fed guppies daily. Guppies in the pred+ condition were continuously exposed to the chemical cues of the cichlid and its prey; no visual cues of predation were used. Although we cannot differentiate between chemical cues of the predator and conspecific guppy chemical cues, both are relevant during predation events. This rearing design mimics natural conditions and has been shown to induce plasticity in multiple traits (Torres-Dowdall et al., 2012; Ruell et al., 2013; Handelsman et al., 2013). Following sexual maturity, fish were housed in 10-litre group tanks. All fish were sexually mature at the time of the experiment, kept on a 12 h:12 h light:dark cycle at CSU, and fed a measured diet twice daily (AM: Tetramin tropical fish flake paste, Tetra Holding, Blacksburg, VA, USA; PM: hatched *Artemia* cysts) with food levels adjusted each week based on the age and size of fish (Reznick, 1982; Reznick et al., 2004). As individuals in the same population share a similar genetic background, this design allows us to discern environmental effects of predation (contrast between individuals from the same population in different rearing conditions) from genetic effects (contrast between populations in a shared laboratory environment).

Scanning electron microscopy

Canal and superficial neuromasts cannot be unequivocally differentiated using DASPEI {2-[4-(dimethylamino)styrl]-*N*-ethylpyridinium iodide; Invitrogen, Grand Island, NY, USA} staining, as canals cannot be visualized. In addition, DASPEI may also stain additional non-neuromast structures, such as taste buds found on the skin of many fishes (e.g. Reutter et al., 1974). We used scanning electron microscopy (SEM) to confirm the identity of putative neuromasts and to differentiate between canal and superficial neuromasts. Whole specimens (*N*=3) were fixed in 2.5% glutaraldehyde, 2% paraformaldehyde in cacodylate buffer for 4 days at 4°C. Fish were dehydrated in a series of increasingly concentrated ethanol baths before being critical-point dried and spur-coated with 5 nm of gold-palladium in a Denton Vacuum Desk II (Moorestown, NJ, USA). SEM samples were imaged in an AMRAY 1620D (Bedford, MA, USA) scanning electron microscope at 10–30 kV acceleration voltage. SEM was performed at the laboratory for Biological Ultrastructure at the University of Maryland.

Neuromast staining and counting

We stained neuromasts in live guppies using the fluorescent vital dye DASPEI (Invitrogen) following a protocol adapted from Wark and Peichel (Wark and Peichel, 2010). DASPEI infiltrates the stereocilia of hair cells *via* an endogenous transporter, becoming trapped inside them and thus specifically labelling the neuromasts (Van Trump et al., 2010). We made a 0.25% DASPEI stock solution by suspending the dye in dH$_2$O and diluted this to a working concentration of 0.025% with conditioned tank water. Guppies were allowed to swim freely in the 0.025% DASPEI solution for 30 min. We then rinsed the guppies in conditioned tank water before anaesthetizing them with 0.02% MS-222 buffered with 0.06% sodium bicarbonate (tricaine methylsulfonate; Acros Organics, Geel, Belgium) until they were motionless and showing shallow gill ventilation. We transferred guppies to a Petri dish containing 0.01% MS-222 for observation and photography. High magnification images were captured using a camera (DP71, Olympus, Center Valley, NJ, USA) connected to a fluorescence stereomicroscope with a green fluorescent protein filter (SZX10,

Olympus). We used these high magnification images to construct a body map of the left side of each fish, from which distinct regions of neuromasts were identified and all neuromasts were counted. Criteria for defining neuromast regions included identity (canal/groove *versus* superficial), spacing (regular *versus* irregular) and arrangement (distinct line *versus* diffuse cluster) of neuromasts, as well as any clear breaks (areas without neuromasts). Our nomenclature reflects the anatomical locations of these groups and is based on previous work in fish in general (e.g. Webb, 1989a) and poeciliids specifically (Rosen and Mendelson, 1960; Guarnieri et al., 1993).

Statistical analyses

Given the challenge of interpreting the functional significance of small-scale changes in neuromast distribution (Coombs et al., 1992; Montgomery et al., 1995; Bleckmann and Zelick, 2009), we ran all our statistical analyses using broadly defined body regions (see Results). We included body size (standard length) as a covariate in all analyses because the number of neuromasts has been shown to increase with increasing body size (Münz, 1979). This covariate was significant in wild-caught but not laboratory-reared individuals, and was thus removed from laboratory-reared analyses.

To examine total neuromast number and neuromast distribution by body region, we used linear mixed models with repeated measures. The dependent variable was the number of neuromasts for each region, with region as a repeated measure. We used Akaike's information criterion indices to select the best-fitting variance structure for the repeated measures, and present here only the best-fitting model (unstructured variance). For wild-caught fish, we included main effects of sex, body region, drainage of origin and predation level in source populations, as well as two- and three-way interactions between body region, drainage and predation level. For laboratory-reared individuals, we included main effects of sex, body region, population of origin and rearing condition, along with two- and three-way interactions between body region, population and rearing condition. Because we found no main effect of sex or its interactions, we removed all higher-order interactions between sex and other main effects and report only these reduced models. We present Type III tests of model effects estimated with restricted maximum likelihood. We used *post hoc* comparisons to identify the effects of population of origin and rearing environment in individual body regions. All contrasts were Tukey adjusted to control for multiple hypothesis testing. To examine count variability in each region, we calculated the index of dispersion, a standardized measure of variability appropriate for count data (Upton and Cook, 2008). We used SAS statistical software version 9.2 (SAS Institute, Cary, NC, USA) for all analyses.

RESULTS
Scanning electron microscopy

Use of SEM revealed the specific location of canals and grooves and allowed us to distinguish between superficial and canal/groove neuromasts. The facial region contained six distinct canal openings in the pre-operculum, as well as obvious grooves rostral and caudal to the eye (Fig. 1A). Consistent with related species (Rosen and Mendelson, 1960; Guarnieri et al., 1993), no canals or grooves were present on the body. The morphology of individual neuromasts was more closely examined using SEM (Fig. 1B–D). While most neuromasts appeared small and circular, we consistently observed a number of larger, oval neuromasts. From SEM, all small, spherical neuromasts were identified as superficial and all larger, oval neuromasts as canal neuromasts located in canals or grooves.
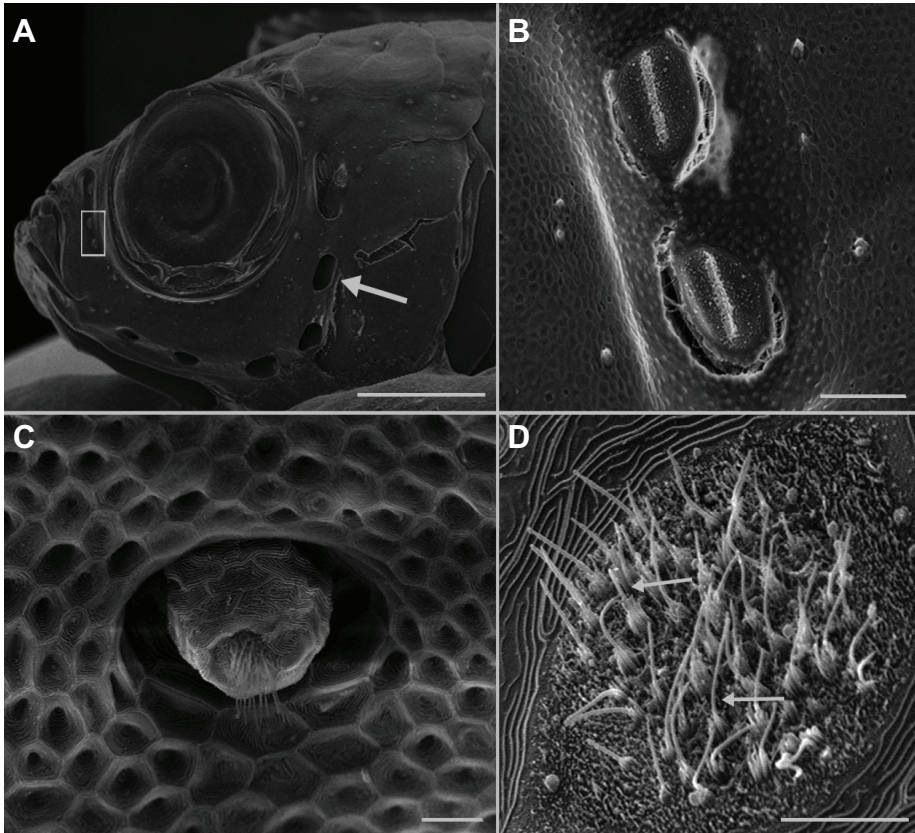
Fig. 1. SEM images of guppy neuromasts and canals. (A) Lateral view of the head. The pre-orbital and post-orbital canals are open grooves, whereas pre-opercular region has enclosed canals with prominent canal pores (arrow). The boxed region in A is magnified in B. (B) High magnification image of neuromasts in the pre-orbital groove. (C) High magnification image of a superficial neuromast from the mandibular line. (D) High magnification of a superficial neuromast from the caudal fin. Arrows in D indicate kinocilia of two of the hair cells. Scale bars, (A) 1000 μm, (B) 100 μm, (C) 10 μm, (D) 1 μm.

General arrangement of neuromasts

Using DASPEI staining, we defined 16 groups of neuromasts in Trinidadian guppies based on their anatomical arrangement and homology with previously defined groups of neuromasts in other poeciliids (Rosen and Mendelson, 1960; Guarnieri et al., 1993). We identified five groups of canal neuromasts in grooves or fully enclosed canals on the head (Fig. 2): three pre-orbital (PR) groove neuromasts, three supraorbital (SO) groove neuromasts, five post-orbital (PO) groove neuromasts, six pre-opercular (OP) canal neuromasts and one mandibular (MG) groove neuromast. These large, oval, canal and groove neuromasts showed no variation in number or location across populations or rearing conditions.

We identified 11 groups of superficial neuromasts on the head and body (Fig. 2): an ethmoid line (ET), a mandibular line (MD), an infraorbital line (IO), an opercular group (OG), an anterior dorsal group (AD), a dorsal group (DO), a dorsal trunk line (Ld), an abdominal group (AB), a midventral trunk line (Mv), a ventral trunk line (Lv) and a caudal fin line (CF). This arrangement of superficial neuromasts was consistent among individuals so that groups of superficial neuromasts could be accurately identified in all individuals, even though there was variation in the number of neuromasts in each of these groups. Trunk canals were completely absent in the guppy; however, the 'replacement lines' made up of superficial neuromasts found on the trunk follow a pattern defined by Webb (Webb, 1989a) as 'multiple' (dorsal and ventral lines, rather than a single trunk line) and 'disjunct' (collections of trunk neuromasts are discontinuous). Neuromast count data by region are summarized in Table 1.

We consolidated the 16 groups of neuromasts we identified into five regions representing major body divisions (Fig. 2): a facial region (F), a dorsal body region (D), a ventral body region (V), and dorsal (Ld) and ventral (Lv) trunk lines. Given a lack of specific knowledge concerning underlying neural pathways in the guppy, the complexity of assigning biological significance to form/function relationships (Coombs et al., 1992; Montgomery et al., 1995), and ambiguities concerning higher order neural processing of lateral line sensory input (Bleckmann and Zelick, 2009), we ran all statistical analyses using these major regions (see below).

Differences in neuromast distribution between populations of wild-caught guppies

In wild-caught guppies, high-predation fish had overall more neuromasts than low-predation fish and the effects of drainage or sex were not significant (Table 2). Predation level ($F_{1,62.5}$=7.99, P=0.0063; Table 2) and the drainage of origin × region interaction ($F_{4,61.5}$=6.90, P=0.0001; Table 2) explained most of the variation. *Post hoc* comparisons (Table 3) revealed that high-predation fish had more neuromasts in the ventral ($F_{1,57.4}$=5.47, P=0.0299; Fig. 3) and dorsal trunk line ($F_{1,67.3}$=7.93, P=0.0064; Fig. 3) regions compared with low-predation fish, and this effect approached significance in the facial region ($F_{1,70}$=3.89, P=0.0524). Fish from the Marianne drainage had more dorsal ($F_{1,58.5}$=16.24, P=0.0002), ventral ($F_{1,57.4}$=4.49, P=0.0385) and dorsal trunk line ($F_{1,67.4}$=4.32, P=0.0414; Fig. 3) neuromasts than those from the Oropuche drainage. Within-group variability was low in all regions (indices of dispersion <1).

Genetic and environmental differences in neuromast distribution in laboratory-reared guppies

We found that laboratory-reared fish from the Taylor low-predation population had more neuromasts than laboratory-reared Guanapo high-predation fish ($F_{1,94.5}$=8.55, P=0.0043), and within populations, pred+ fish had more neuromasts than pred– fish ($F_{1,94.5}$=7.66, P=0.0068; Table 4). We found no differences in the total number
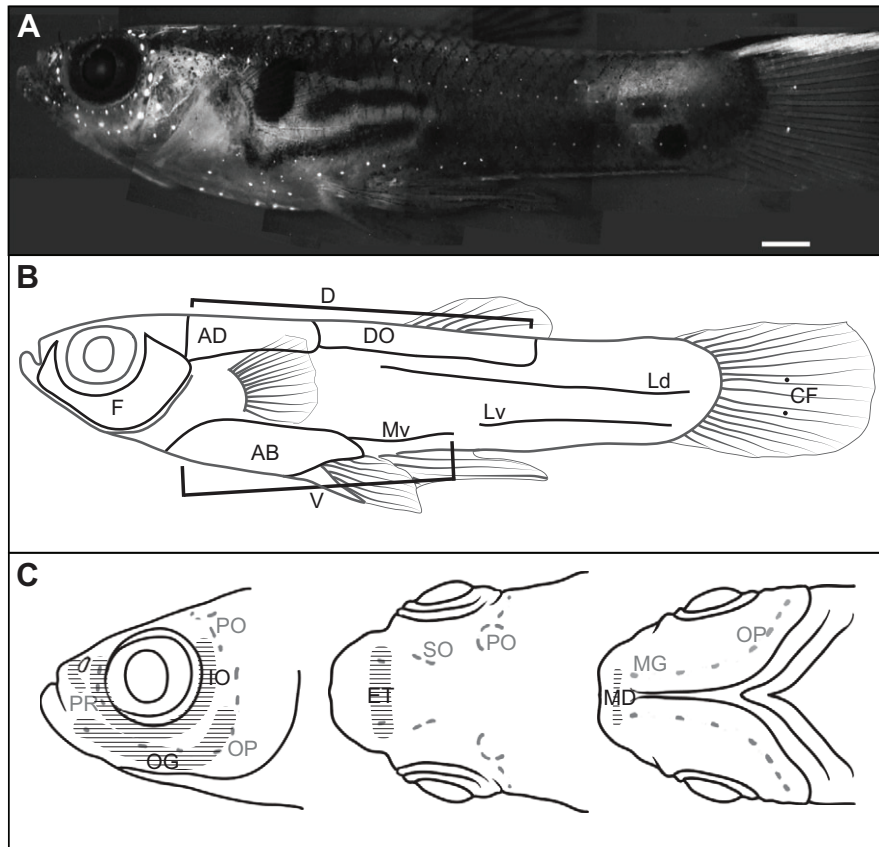
Fig. 2. (A) Photomontage showing DASPEI staining on the left side of a representative male guppy. Scale bar, 1 mm. (B) Schematic representation of neuromast groups and major regions on the body of a male guppy (regions do not vary by sex). Detail of facial region F is shown in C. (C) Facial region from lateral, dorsal and ventral perspectives, respectively. Canal and groove neuromasts are shown as grey dots and their names are also in grey. Line shading indicates locations of groups of superficial neuromasts whose names are in black. AB, abdominal group; AD, anterior dorsal; CF, caudal fin group; D, dorsal region; DO, dorsal group; ET, ethmoid line; F, facial region; Ld, dorsal trunk line; Lv, ventral trunk line; MD, mandibular group; MG, mandibular groove; Mv, midventral line; OG, opercular group; OP, pre-opercular canal; PO, post-orbital groove; PR, pre-orbital groove; SO, supraorbital groove; V, ventral region.

of neuromasts between sexes. Effects of population of origin and rearing environment differed by body region (population×region: $F_{4,85}$=3.37, P=0.0130; rearing×region: $F_{4,85}$=7.66, P<0.0001; Table 4). *Post hoc* comparisons (Table 5) revealed that fish from the Taylor low-predation population had more neuromasts in dorsal ($F_{1,91.3}$=14.47, P=0.0003) and facial ($F_{1,96.8}$=5.06, P=0.0268; Fig. 4) regions compared with fish from the Guanapo high-predation population. Fish reared in the presence of predator chemical cues had significantly more neuromasts in facial regions compared with fish reared without predator chemical cues ($F_{1,96.8}$=22.81, P<0.0001; Fig. 4), and this effect approached significance in the dorsal trunk line ($F_{1,92.5}$=3.37, P=0.0694). Within-group variability was low in all regions (indices of dispersion <1).

DISCUSSION

We found that wild-caught guppies exhibited differences in superficial neuromasts based on predation level and drainage of origin. These differences were mirrored by both genetic and environmental variation in laboratory-reared fish. We conclude that both genetic and environmental factors influence neuromast number and distribution, with some body regions more prone to variation than others. We discuss possible adaptive and non-adaptive explanations for genetic and environmental differences in lateral line morphology. We focus our discussion on behavioural and developmental traits that are known to differ based on predation pressure for two primary reasons. First, although additional environmental variables covary with predation regime, differences in predation pressure are thought to be the main driver of adaptive divergence between high- and low- predation population pairs in the wild (reviewed in Magurran, 2005). Second, whereas additional environmental variables may contribute to genetic divergence,

environmentally mediated differences in laboratory-reared guppies are most likely a response to predator cue exposure, as all other environmental variables are controlled in the laboratory.

Variation in neuromast distribution is restricted to superficial neuromasts

We wanted to distinguish variability in canal *versus* superficial neuromasts. Based on SEM, large, oval neuromasts lay deep in grooves or fully enclosed canals. Because no small circular neuromasts were found in canals or grooves, we identified the small, circular neuromasts in DASPEI staining as superficial neuromasts. Consistent with other species (e.g. Münz, 1979), canal neuromasts are larger and more elongated than superficial neuromasts in guppies. We found remarkable consistency between populations in the number and location of the larger, oval neuromasts identified as canal neuromasts. Thus all the variability in lateral line morphology reported here arose from differences in superficial neuromasts. This lack of variation of canal neuromasts is consistent with the well-established idea that superficial neuromasts are generally more flexible in their number and location than canal neuromasts (e.g. Webb, 1989a; Webb and Noden, 1993; Webb and Shirey, 2003).

The reduced canal system that we observed in the guppy is consistent with previous descriptions in related genera (Rosen and Mendelson, 1960; Guarnieri et al., 1993; Wildekamp and Malumbres, 2004; Lucinda et al., 2005), and with the observation that many teleost fish species have reduced canal systems in which series of canal neuromasts are replaced by superficial neuromasts (e.g. Webb, 1989a). Such reduced canal systems are thought to increase lateral line sensitivity to water flows (Dijkgraaf, 1963), and are particularly common in fish that live in low-flow environments

Table 1. Mean (±s.d.) number of superficial neuromasts by body region in wild-caught and laboratory-reared guppies

Drainage/Population	Predation/Treatment	Sex	N	AB	AD	CF	DO	ET	IO	Ld	Lv	MD	MV	OG	Total
Wild-caught															
Marianne	Low-predation	F	13	18.5±2.3	10.2±1.2	2.3±1.1	12.0±2.2	2.8±0.4	25.5±2.4	11.7±2.9	10.2±2.0	2.9±0.3	6.6±1.6	17.0±1.9	119.5±8.4
		M	9	16.6±2.5	8.7±0.9	1.9±0.3	8.8±2.8	2.7±0.7	22.7±2.1	13.1±1.6	9.4±2.3	2.6±0.7	7.0±2.1	18.4±2.3	114.0±11.2
	High-predation	F	11	18.8±2.0	11.1±1.1	2.2±0.6	11.0±1.8	3.0±0.4	25.2±2.1	13.9±2.7	8.7±2.0	2.9±0.6	9.1±1.6	19.5±2.3	124.6±7.7
		M	7	17.4±1.8	9.9±1.1	2.3±0.8	11.1±1.1	3.0±0.0	25.0±1.8	14.3±1.1	9.7±1.0	2.7±0.5	8.0±1.3	19.7±1.3	123.1±4.3
Oropuche	Low-predation	F	13	15.9±3.8	9.3±1.4	2.3±0.6	9.6±2.6	2.9±0.3	24.5±2.7	13.5±3.1	8.5±1.1	3.1±0.5	7.8±1.9	17.8±2.7	115.9±11.0
		M	7	15.3±2.2	9.0±0.9	2.2±1.0	7.7±1.6	3.0±0.0	23.1±3.3	13.6±1.5	8.9±1.5	2.7±0.8	8.0±1.5	19.5±3.0	114.5±6.0
	High-predation	F	7	17.3±2.7	8.7±3.1	3.0±1.5	9.0±3.2	2.9±0.4	25.0±4.9	14.9±1.5	10.6±2.4	2.9±0.4	7.1±1.5	18.9±3.7	118.3±17.4
		M	8	16.9±2.9	9.9±1.6	2.3±0.7	7.6±1.8	3.0±0.0	23.5±2.0	15.0±2.0	10.3±2.4	2.8±0.5	7.4±1.3	19.0±2.4	118.4±6.5
Laboratory-reared															
Guanapo	Pred–	F	15	14.8±3.3	9.9±2.0	2.1±0.8	6.7±2.5	2.7±0.6	20.7±3.3	15.1±2.2	10.0±1.7	2.7±1.0	6.9±1.7	17.9±2.6	109.5±12.6
		M	15	16.8±4.9	9.8±2.2	2.1±0.5	7.5±2.7	2.6±0.5	20.2±3.8	13.7±2.6	9.5±1.9	2.6±0.5	7.1±1.9	17.3±3.2	109.1±15.6
	Pred+	F	10	18.2±2.4	10.7±0.9	2.8±1.0	9.0±2.1	3.0±0.0	24.2±1.7	15.0±2.8	10.2±1.6	3.0±0.0	7.6±1.4	20.7±3.4	124.4±9.1
		M	10	16.4±3.0	9.8±2.5	2.4±0.8	6.3±3.2	2.9±0.3	22.7±3.1	13.8±4.2	10.1±2.0	3.0±0.0	8.7±2.4	20.2±2.1	115.4±18.3
Taylor	Pred–	F	15	15.4±2.9	10.7±1.6	3.6±1.1	9.9±1.7	2.9±0.2	23.9±2.3	16.2±2.1	10.2±1.5	2.8±0.4	9.4±2.2	19.5±3.4	124.6±9.6
		M	15	15.1±3.3	10.2±2.6	2.0±0.8	8.3±2.3	2.9±0.3	21.3±3.3	15.3±1.9	10.1±1.2	2.8±0.8	9.3±2.1	17.3±1.9	114.7±9.6
	Pred+	F	10	16.3±2.1	11.0±1.9	3.0±1.1	9.4±2.2	3.0±0.0	23.5±2.1	15.0±1.2	9.9±1.1	2.8±0.4	10.3±1.3	20.6±2.1	124.8±5.9
		M	10	15.0±5.3	11.3±1.9	2.4±0.9	8.6±1.3	3.0±0.0	24.0±1.6	12.9±2.2	9.9±0.9	3.0±0.0	9.8±2.6	21.8±1.7	121.7±8.8

AB, abdominal group; AD, anterior dorsal group; CF, caudal fin line; DO, dorsal group; ET, ethmoid line; IO, infraorbital line; Ld, dorsal trunk line; Lv, ventral trunk line; MD, mandibular line; MV, midventral line; OG, opercular group.

Table 2. Effects of sex, drainage and population of origin, body region and their interactions on neuromast distribution in wild-caught guppies

Source of variance	d.f.	F	P
Drainage	1, 62.5	3.60	0.0624
Predation	1, 62.5	7.99	0.0063
Sex	1, 67.0	0.26	0.6148
Neuromast region	4, 61.8	26.37	<0.0001
Drainage × Predation	1, 62.3	0.01	0.9051
Drainage × Region	4, 61.5	6.90	0.0001
Predation × Region	4, 61.7	1.53	0.2041
Drainage × Predation × Region	4, 61.5	2.42	0.0580

Main effects and their interactions were evaluated using a mixed model with regions as a repeated measure within individual.

(e.g. Coombs et al., 1992; Montgomery et al., 1995). Although poeciliids vary in the presences of canals and number of canal pores [for a survey of several species, see Rosen and Mendelson (Rosen and Mendelson, 1960)], the locations of canals and grooves in the guppy are consistent with those in other poeciliids.

The lack of variation in canal neuromast number and location across populations could result from a diversity of processes, including strong stabilizing selection, developmental constraints and phylogenetic history. Canal neuromasts detect high-frequency motion (Bleckmann, 1993), for example the movements of small prey items (Montgomery et al., 1995). Small invertebrates are a high-quality food source for guppies (Zandonà et al., 2011), and the ability to detect small, moving prey may act as a strong selective force constraining changes in the number and/or location of canal neuromasts in guppies from all populations. In addition, developmental constraints may contribute to the lack of variation in canal neuromasts, as canal neuromasts are patterned early in development (e.g. Blaxter, 1987; Gompel et al., 2001) and may participate in dermal bone formation (Webb, 1989b; Webb and Noden, 1993).

Differences among wild-caught fish in superficial neuromast distribution

We found that the total number and distribution of superficial neuromasts by body region differed between drainages and between high- and low-predation populations in wild-caught guppies. Fish from the Marianne drainage had more superficial neuromasts compared with those from the Oropuche drainage, and high-predation populations had more neuromasts compared with their low-predation counterparts in each drainage. Guppies from the Marianne drainage had relatively more neuromasts in dorsal and ventral regions, and high-predation guppies had relatively more neuromasts in ventral and dorsal trunk line regions, with the effect of predation also approaching significance in the facial region (Fig. 5).

As has been suggested for other species, variation in neuromast number and distribution may be directly tied to adaptive behavioural differences [*Gasterosteus aculeatus* (Wark and Peichel, 2010); *Astyanax mexicanus* (Yoshizawa et al., 2010); and *Pungitius pungitius* (Trokovic et al., 2011)]. For example, neuromasts are used to maintain fish spacing in schooling or shoaling fish (e.g. Partridge and Pitcher, 1980; Faucher et al., 2010). High-predation guppies shoal more often and more tightly than low-predation guppies (Seghers, 1974; Magurran and Seghers, 1990; Seghers and Magurran, 1995; Huizinga et al., 2009), and having more neuromasts could help high-predation fish maintain consistent proximity during

Table 3. *Post hoc* comparisons of neuromast number by body region in wild-caught guppies

Neuromast region	Drainage			Predation		
	d.f.	F	P	d.f.	F	P
F	1, 69.9	0.27	0.6049	1, 70.0	3.89	0.0524
D	1, 58.5	16.24	**0.0002**	1, 58.7	1.58	0.2135
Ld	1, 67.4	4.32	**0.0414**	1, 67.3	7.93	**0.0064**
V	1, 57.4	4.49	**0.0385**	1, 57.4	5.47	**0.0229**
Lv	1, 69.0	0.13	0.7218	1, 68.9	0.74	0.3932

All *P*-values were Tukey adjusted to control for multiple-testing error. Bold values indicate significance at *P*<0.05.
F, facial region; D, dorsal body region; Ld, dorsal trunk line; V, ventral body region; Lv, ventral trunk line.

shoaling. A second behavioural difference between high- and low-predation guppies is water column use. High-predation guppies spend more time at the top of the water column to avoid piscivorous predators attacking from below (Seghers, 1974; Botham et al., 2006; Torres-Dowdall et al., 2012). A greater number of ventral neuromasts in high-predation fish could be particularly useful as the majority of relevant hydrodynamic stimuli come from below in this context. Finally, high- and low-predation guppies differ in the frequency, duration and intensity with which they perform courtship displays (Houde, 1997; Magurran, 2005; O'Steen et al., 2010). The stereotypical courtship display of male guppies is the sigmoid display, in which a male positions himself perpendicular in front of a female, assumes a curved posture, and rapidly quivers his body (reviewed in Houde, 1997). Sigmoid displays are thought to generate

a series of small pressure waves that females may monitor to assess male quality (Houde, 1997; Magurran, 2005), and sigmoid locomotor performance correlates with survival ability in guppies (O'Steen et al., 2010). Additional neuromasts – in particular in the facial region – may enhance accurate detection of pressure waves generated during sigmoid displays. In sum, differences in shoaling, water column use and courtship behaviour, in combination with differences in neuromast number and distribution, may be of adaptive significance under divergent predation regimes.

Whereas behavioural differences between high- and low-predation guppies tend to be similar across population pairs, high-predation sites in different drainages differ substantially in the make-up of their predator communities (reviewed in Magurran, 2005). The Marianne drainage has a high incidence of diurnal prawn
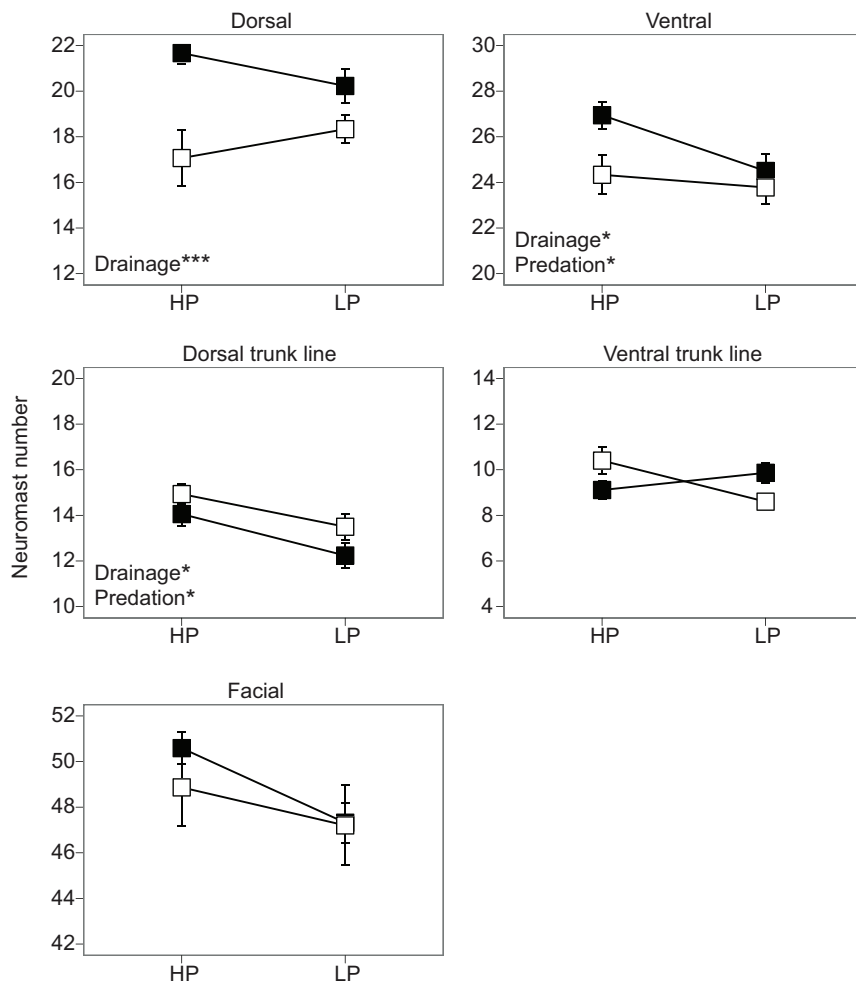

Fig. 3. Comparisons of neuromast number by region in wild-caught guppies. White shapes represent the Oropuche drainage; black shapes represent the Marianne drainage. Where present, significant effects are indicated in the lower left-hand corner (*P<0.05; ***P<0.0001). Error bars indicate ±s.e.m.

Table 4. Effects of sex, population of origin, rearing environment, body region and their interactions on neuromast distribution in laboratory-reared guppies

Source of variance	d.f.	F	P
Population	1, 94.5	8.36	0.0043
Rearing	1, 94.5	6.22	0.0143
Sex	1, 92.5	0.02	0.8897
Neuromast region	4, 85.0	976.26	<0.0001
Population × Rearing	1, 85.0	1.91	0.1700
Population × Region	4, 85.0	3.37	0.0130
Rearing × Region	4, 85.0	7.66	<0.0001
Population × Rearing × Region	4, 85.0	0.80	0.5264

Main effects and their interactions were evaluated using a mixed model with regions as a repeated measure within individual.

predators (genus *Macrobrachium*) compared with other drainages (reviewed in Magurran, 2005), and predator community composition contributes to genetic and environmentally mediated differences in behaviour, morphology and life history among drainages (Seghers, 1974; Rodd and Reznick, 1991; Magurran, 2005). Differences in neuromast distribution between drainages may thus reflect adaptation to different predator communities. We do not currently know how changes in neuromast number and distribution influence lateral line information processing and behaviour; however, our data implicate neuromasts in the dorsal and ventral regions as candidates for future research.

Although much of the variation observed in guppies is associated with lifetime exposure to and/or evolutionary history with predators (Endler, 1995; Houde, 1997; Magurran, 2005; Reznick et al., 2008), adaptive explanations must also consider the potential effects of other covarying environmental differences (Coombs et al., 1992). A suite of environmental factors covaries with predation pressure, and the multivariate nature of these environments results in a diversity of selection pressures that may directly or indirectly influence neuromast distribution. For example, differences in food availability and food types (Reznick et al., 2001; Zandonà et al., 2011), water flow characteristics (Reznick et al., 2001), body shape (Alexander et al., 2006) and developmental timing (e.g. Reznick et al., 1990; Arendt and Reznick, 2005) between high- and low-predation populations could contribute to changes in neuromast number and distribution (Rosen and Mendelson, 1960; Münz, 1979; Coombs et al., 1992; Levin, 2010).

Additional non-adaptive forces that may play a role in the guppy system include genetic drift and developmental constraints. Comparative genetics suggest that low-predation populations were founded by a small number of individuals and have reduced genetic variation (e.g. Crispo et al., 2006; Alexander et al., 2006), which increases the potential for the fixation of alleles and traits due to genetic drift. Developmental constraints could result from reduced genetic variation, coupling between neuromast development and other morphological characters, or could be due to the robustness of developmental programmes related to neuromast patterning. Regardless of the precise mechanism, such developmental constraints may influence patterns of variation in neuromast distribution by restricting variability in some body regions but not others.

Influence of rearing environment and population of origin on superficial neuromast distribution

The contribution of environmentally mediated variation in lateral line morphology has not been explicitly considered in other species. We demonstrated substantial effects of rearing environment on neuromast number and distribution by body region in laboratory-reared fish. These results suggest that some differences in wild-caught fish may also be environmentally mediated, due to the presence of predators or other features of the complex natural environment (Grether et al., 2001; Reznick et al., 2001; Zandonà et al., 2011). Below, we focus on the effects of predator cues on lateral line morphology, as other environmental factors are controlled in the laboratory.

Our breeding design allowed us to disentangle genetic and environmental influences of predation on lateral line morphology. We found that both population of origin and rearing environment influenced patterns of neuromast distribution. We observed differences between high- and low-predation populations primarily in the pred– rearing environment, which mimics the derived environment. These results suggest that the predator-induced increase in the number of neuromasts in certain regions is conserved across populations, and when exposed to the predator cue both populations exhibit a high-predation phenotype. As a result, genetically based population differences are only revealed in the absence of predator cues. Recent work demonstrates a similar pattern for other traits in guppies: fish from low-predation source populations revert to ancestral high-predation phenotypes for life history, morphological and behavioural traits when raised in the presence of chemical predator cues (Torres-Dowdall et al., 2012). Given that guppies are small prey fish, retaining the ancestral ability to respond plastically to predator cues is likely a highly conserved trait, but more studies are required to understand the mechanisms by which this developmental plasticity is manifested.

One possible mechanism underlying environmentally induced differences in lateral line morphology may be changes in developmental timing. While guppies from high-predation populations grow faster and mature earlier than their low-predation counterparts (Arendt and Reznick, 2005), the plastic response of guppies raised with predator cues is a slower growth rate compared with those raised without predator cues (Handelsman et al., 2013). Alterations in developmental timing are one appealing explanation for environmentally mediated changes in lateral line morphology,

Table 5. *Post hoc* comparisons of neuromast number by body region in laboratory-reared guppies

Neuromast region	Population			Rearing		
	d.f.	F	P	d.f.	F	P
F	1, 96.8	5.06	**0.0268**	1, 96.8	22.81	**<0.0001**
D	1, 91.3	14.47	**0.0003**	1, 91.2	1.55	0.2159
Ld	1, 92.5	0.74	0.3913	1, 92.5	3.37	0.0694
V	1, 98.0	2.73	0.1016	1, 98.0	2.13	0.1480
Lv	1, 94.7	0.06	0.8123	1, 94.7	0.07	0.7898

All *P*-values were Tukey adjusted to control for multiple-testing error. Bold values indicate significance at *P*<0.05.
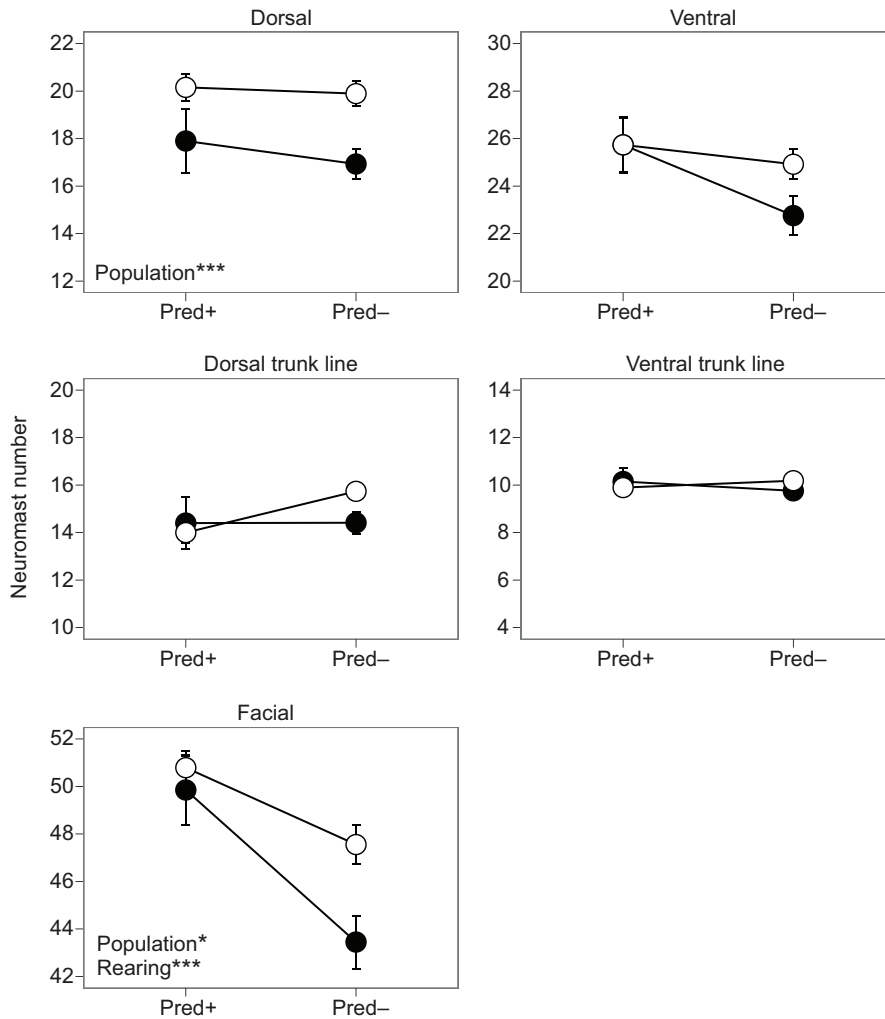See Table 3 footnote for abbreviation definitions.

Fig. 4. Comparisons of neuromast number by region in laboratory-reared guppies. White shapes represent Taylor low-predation source population; black shapes represent Guanapo high-predation source population. Where present, significant effects are indicated in the lower left-hand corner (*P<0.05; ***P<0.0001). Error bars indicate ± s.e.m.

as most differences in lateral line morphology are thought to rely on heterochrony (Coombs et al., 1992), and experimentally induced heterochrony has been shown to influence neuromast number in other species (Levin, 2010). Future studies are needed to evaluate whether environmental influences on developmental timing do indeed alter neuromast distribution in guppies and whether genetic differences among populations depend on faster development in high-predation guppies compared with low-predation guppies (e.g. Arendt and Reznick, 2005).

Given the wide range of behaviours influenced by lateral line sensory input – including, but not limited to, examination of novel objects (Teyke, 1990; Burt de Perera, 2004), detection of prey items (Hoekstra and Janssen, 1985; Janssen et al., 1999; Yoshizawa et al., 2010), monitoring of conspecifics (Partridge and Pitcher, 1980; Partridge et al., 1980; Satou et al., 1994; Faucher et al., 2010) and predators (McHenry et al., 2009), and maintenance of position in flowing water (Sutterlin and Waddy, 1975; Montgomery et al., 1997) – variation in lateral line morphology is likely to have behavioural consequences. Both evolutionary history with predators and environmental exposure to predator cues increase shoaling behaviour (Huizinga et al., 2009), and differences in water column use are also environmentally mediated (Torres-Dowdall et al., 2012). The capacity to increase neuromast number in response to predator cue exposure may have functional consequences for shoaling behaviour and predator avoidance and thus be subject to natural selection in high-predation localities.
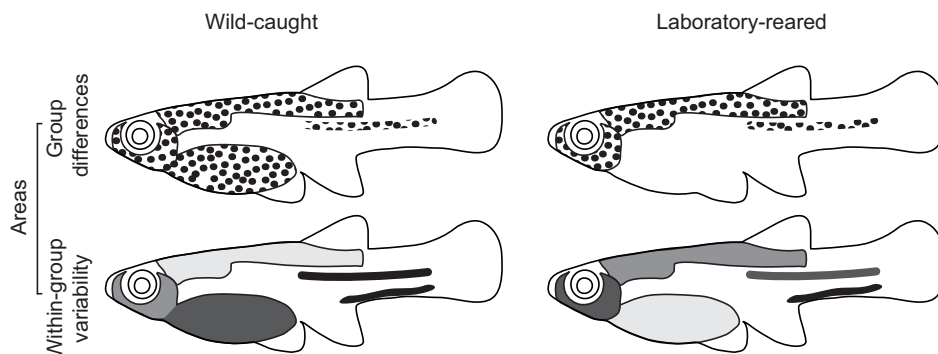

Fig. 5. Differences in neuromast number occur in the same body regions across populations and rearing conditions in laboratory-reared and wild-caught guppies (top row; shading indicates group differences). Overall within-group variability is low but varies by body region (bottom row; darker colours indicate less variability).

Developmental constraints and genetic drift may also shape lateral line morphology between populations. Examining patterns of variability in neuromast number within and among regions on the head and trunk can help us distinguish between selection, developmental constraints and genetic drift as each hypothesis makes different predictions concerning within- and between-group variability. Differences in neuromasts between high- and low-predation populations could be adaptive responses to selection pressures common across low-predation localities and distinct from selection pressures in high-predation environments. If neuromast differences are indeed adaptive responses, we expect low within-population variability in neuromast number as a consequence of selection on this trait, and similar patterns when we compare high- and low-predation population pairs across drainages, as is typical of many traits in guppies (reviewed in Magurran, 2005). Indeed, all regions show relatively low within-group variability, and some regions show consistent, directional differences among groups in both wild-caught and laboratory-reared fish (e.g. dorsal region; Fig. 5). If, in contrast, developmental constraints restrict variability, we expect low variation both within and among groups, consistent with our observations in other regions (e.g. ventral trunk line; Fig. 5) and in canal neuromasts. Finally, if drift is a driving force we expect low within-group variation, especially in low-predation populations, which have smaller effective population sizes (e.g. Crispo et al., 2006; Alexander et al., 2006). With drift, genetic differences may exist, but we do not expect them to be consistent among high- and low-predation population pairs from different drainages. Although we do not see patterns exclusively associated with genetic drift, differences in response magnitude among drainages may be a consequence of drift. Combining results from laboratory-reared and wild-caught fish, we conclude that (1) selection has likely led to parallel patterns of variation in neuromast number and distribution among populations, but (2) genetic drift may contribute to differences in response magnitudes, and (3) developmental constraints restrict variability in some regions.

Conclusions

We found genetically and environmentally mediated differences in neuromast number and distribution in guppies. Although effect direction and magnitude differed, we consistently observed variation in facial, dorsal and ventral regions in laboratory-reared and wild-caught fish. These regions may be repeatedly targeted because they are particularly important in specific behaviours relevant to predator avoidance, courtship behaviour or feeding, and/or they are not subject to developmental constraints that may restrict variation in other regions. Additional studies are required to understand the forces driving variation in some regions but not others, both within and among species.

To the best of our knowledge, this is the first time that environmental exposure to an ecologically relevant stimulus has been shown to mediate variation in lateral line morphology. The substantial contribution of environmental factors we see here offers one explanation for discrepancies between wild-caught and laboratory-reared fish observed in previous studies (e.g. Trokovic et al., 2011). A better understanding of the complex interplay between genetic and environmental forces shaping this sensory system will shed light on processes underlying lateral line evolution.

LIST OF ABBREVIATIONS

AB	abdominal group
AD	anterior dorsal group
CF	caudal fin line
D	dorsal body region
DASPEI	2-[4-(dimethylamino)styrl]-N-ethylpyridinium iodide
DO	dorsal group
ET	ethmoid line
F	facial region
GH	Guanapo high-predation
HP	high-predation
IO	infraorbital line
Ld	dorsal trunk line
LP	low-predation
Lv	ventral trunk line
MD	mandibular line
MG	mandibular groove
MH	Marianne high-predation
ML	Marianne low-predation
Mv	midventral trunk line
OG	opercular group
OH	Oropuche high-predation
OL	Oropuche low-predation
OP	pre-opercular canal
PO	post-orbital groove
PR	pre-orbital groove
Pred–	rearing environment without predator chemical cues
Pred+	rearing environment with predator chemical cues
SEM	scanning electron microscopy
SO	supraorbital groove
TL	Taylor low-predation
V	ventral body region

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