J Ethol (2017) 35:109–119
DOI 10.1007/s10164-016-0498-0

ARTICLE

Social plasticity in non-territorial male African cichlid fish *Astatotilapia burtoni*

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Received: 11 May 2016 / Accepted: 25 September 2016 / Published online: 27 October 2016

Abstract The African cichlid fish *Astatotilapia burtoni* represents a valuable model system for studies of social decision-making due to its socially mediated phenotypic plasticity. The males reversibly transition in social status from reproductively dominant and territorial (DOM) to submissive and non-territorial (SUB). Males are traditionally categorized into these two behavioral phenotypes by observational scoring. There is evidence, however, that this dichotomous categorization might not capture the behavioral plasticity displayed by individuals transitioning between SUB and DOM status. To test this concept, we used focal observations of intrasexual conflict behavior in fish communities combined with a modified analysis of the ethogram typically used in *A. burtoni*. Results revealed a cluster of males close to the crossover point between SUB and DOM status as defined by the traditional dominance index. These intermediate males showed the highest frequency of intrasexual conflict behaviors, distinct behavioral responses to threats, and body pigment signaling displays that distinguish them from prototypical SUBs and DOMs. As such, our results provide a noninvasive behavioral metric to categorize *A. burtoni* males into three groups, thus further capturing the complex social dynamic of this model organism.

Keywords Social behavior · Reversible phenotype · Dominance · Territoriality · Intrasexual competition

Introduction

Competition for resources, including mating opportunities, shelter, and food access, has a strong influence on many experiential and fitness-related/life history traits of individuals in a social system (e.g., Erickson 1967; Genner et al. 1999; Maher and Lott 2000; Clement et al. 2005). Variation based on success in these types of competition is often used to characterize individuals in terms of community hierarchy, territory ownership, reproductive opportunity, and cooperative contributions (e.g., Baerends and Baerends-Van Roon 1950; Rowland 1997; Hofmann et al. 1999; Oliveira et al. 2002; Chen and Fernald 2011). These characteristics, as well as traits such as sex, age, and reproductive status, contribute to determining social status and often are used as a comparative baseline in research on many aspects of social behavior in many taxa (e.g., Dittus 1977; Ågren 1984; Stutchbury 1994; Hofmann et al. 1999; Clement et al. 2005; Chen and Fernald 2011).

The African cichlid fish *Astatotilapia burtoni* (Günther 1894) represents a well-characterized vertebrate model system for examining the effects of the social environment on behavior (as well as physiology), as males reversibly switch phenotype in strong association with social cues (e.g., Fernald 1977; Greenwood and Fernald 2004; Burmeister et al. 2005; Clement et al. 2005; Renn et al. 2009). Males transition in social status from being territorial (DOM) and monopolizing reproduction to being non-territorial (SUB) without functional gonads, and vice versa (e.g., Fernald 1977; Renn et al. 2009). In

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a social community, *A. burtoni* congregate around a lek-like set of territories. The territories are occupied by DOMs while SUB males and females spend the majority of their time shoaling together (Fernald 1977; Fernald and Hirata 1977; Ferno 1987; Korzan et al. 2008; Renn et al. 2009).

Traditionally, a dominance index (DI) score is calculated for individual *A. burtoni* to characterize social status (Korzan et al. 2008; Renn et al. 2009). Specifically, agonistic, territorial, and reproductive behaviors are added and scored as positive values. Submissive behaviors such as fleeing are scored as negative values. The total combined value is used to calculate the DI. Due to the nature of the behaviors scored, the DI inherently reflects the outcome of agonistic encounters: submissive behaviors reflect a losing outcome for the individual by definition. To the best of the authors' knowledge, this index is currently the only noninvasive metric used to characterize male types in this species. The DI divides males by classifying them either as DOM (positive value) or SUB (negative value) with a DI of zero used as the crossover or point of division between these two groups. However, there is evidence that this dichotomy might not capture the behavioral and physiological plasticity displayed when it comes to individuals transitioning between SUB and DOM status (Fernald 1977; Fernald and Hirata 1977; Hofmann et al. 1999; Desjardins et al. 2012). Some individuals which do not show the traits of full DOMs still do exhibit a more DOM-like hormonal suite as compared to SUBs, and observation in the wild showed that some SUBs and not fully DOM individuals also engage in conflicts that do not result either in fleeing or aggressive response (Fernald and Hirata 1977; Hofmann et al. 1999). Additionally, males ascending to DOM status appear to monitor aggression among other males, with attention directed up the hierarchy (Desjardins et al. 2012).

In sum, the physiological and behavioral traits exhibited by transitioning males suggest that the social pressures associated with ascent to or descent from DOM status are possibly distinct enough to be considered as an additional male phenotype (Hofmann et al. 1999; Oliveira et al. 2002; Korzan et al. 2008). Thus, we predict that males of uncertain territorial status (i.e., near-zero DI score) are likely to behave not only more similarly to one another as compared to either prototypical, fully established DOMs or to prototypical, fully SUBs, but also with a distinct behavioral suite more reflective of a greater potential range of outcomes to intrasexual conflict. Our results show that a modified version of the traditional DI ethogram, and an alternative quantification, the "conflict index" (CI), can be used for a noninvasive categorization of such a third male phenotype within *A. burtoni* communities.

Methods

Subjects

Two communities, each containing from ten to 12 male and five female lab-strain *A. burtoni* were used for behavioral observations. Individuals were originally obtained from the Hofmann lab at the University of Texas, Austin, but have been bred in the Preuss lab for several generations. They were housed in acrylic tanks (30 × 30 × 60 cm) with flow-through conditioned water maintained at pH 8.5 ± 0.2, 27 ± 0.2 °C, 550–650 µS/cm conductivity to ensure a constant environment appropriate to *A. burtoni*. Terra-cotta pots were provided to permit males to form territories. Cichlids were fed daily using a standard aquarium cichlid diet. The room was kept on a 12:12-h light/dark cycle. These conditions, specifically tank size, population density, and subject strains are standard in research on *A. burtoni* social plasticity (e.g., Hofmann et al. 1999). Although the body lengths of individuals used in this study were not measured (see rationale below) other studies in our laboratory show that the standard body length of experimental males from the laboratory population typically ranges between 5.5 and 8 cm. Tank size and number of animals per tank were chosen to match previous studies in *A. burtoni* (e.g., Neumeister et al. 2010). Each tank typically contained from three to five established territories.

Behavioral focal observation

Ethogram recordings were taken using a continuous-sampling method according to previous categorization of male social status in this species (Baerends and Baerends-Van Roon 1950; Fernald 1977; Burmeister et al. 2005; Fox et al. 1997; Renn et al. 2009). Each male was observed as a focal animal for 10 min twice weekly between 1000 and 1400 hours for a total of 16 observations. Before each initial observation within a session, a 10-min period was allowed to acclimate the fish to the presence of the observer. Communities were assembled and undisturbed for weeks, without focal observation, prior to this experiment. To minimize interference with the behavior of individuals and the development of the social system, body size, gonadal growth and hormonal status were not recorded. The existing ethogram was modified to accommodate additional categorization as explained below (additionally, see Table 1). Prior to the experiment, individuals were superficially marked for identification using small patterns applied with Alcian Blue 8GX dye. Although males engaged in physical confrontations, no serious injury was observed during the study. All procedures were conducted according to the guidelines of the Hunter College City

Table 1 Definitions of male behaviors

Behavior	Definition
Confrontations	Includes all agonistic encounters including fleeing; excludes shoaling and pigment displays
Threats	Includes all aggressive behaviors initiated by the focal fish; excludes fleeing, ignoring a threat, shoaling and pigment displays
Reproductive behaviors	Spawning, nest-building, and courtship
Bite (+)	Physical contact with mouth closing on other individual
Poke (+)	Physical contact, not resulting in bite
Chase (+)	Shortening distance to target abruptly, independent of target reaction
Threat display (+)	Back-and-forth movement often accompanied by opercular flaring, typically oriented towards other males
Border threat (+)	Same as threat display but occurs at the border of territory, or "scrape"
Carousel (+)	Dyadic circular movement of opponents with each individual shortening distance to opponent's tail
Ignore threat	Threatened individual does not respond with freezing, flight or other displacement, and remains swimming/floating without response visible to observer
Flee (−)	Increasing distance abruptly as response to chase or poke
Shoal	In close proximity to two or more conspecifics (typically from one to two body lengths)
Lachrymal stripe display	Melanistic pigment, vertical black stripe on either side of the head in lachrymal area (Desjardins and Fernald 2008)
Vertical stripe display	Melanistic pigment, on either side of the body along rib area (Fernald and Hirata 1979)

Plus sign Dominant behavior, *minus sign* submissive behavior

University of New York (CUNY) Institutional Animal Care and Use Committee (IACUC).

Dominance and conflict indices

During each focal observation session, the DI was calculated by subtracting the number of submissive behaviors (−) i.e., fleeing, from the number of dominant behaviors (+) i.e., threats, reproductive behaviors, exhibited by a given male (Table 1) (Fernald 1977; Fox et al. 1997). The mean DI score of each male was then calculated from the individual-session DI scores throughout the 8-week observation period. To test for grouping patterns within the arrays of behaviors salient to territorial status ($n = 22$) we applied agglomerative hierarchal cluster analyses using Ward's minimum variance method (JMP Pro 11). For validation, behavioral patterns between the clusters were compared with one-way ANOVA followed by Tukey post hoc tests when appropriate, and with simple linear regressions (JMP Pro 11).

We also developed an alternative analysis of the above ethogram that highlights the overall number of intrasexual agonistic encounters of males: the CI. The CI is the sum (rather than the difference) of submissive and threat behaviors, both having a positive value, and excludes reproductive behaviors (see Results for rationale).

Results

Behavioral phenotypes in *A. burtoni*

To identify possible behavioral subtypes within *A. burtoni* communities we applied a combined cluster analysis that used three behavioral variables related to territorial status: threat, flee, and shoal. Figure 1a presents a dendrogram of the clustering sequence of 22 males that suggests a three-cluster division as the most parsimonious, as indicated by the distance scree plot (Fig. 1b). Specifically, intracluster homogeneity (the similarity of scores within a cluster) and intercluster heterogeneity (difference between scores among clusters) are maximized by a three-cluster solution as indicated by a sharp rise in data variance following the third segment in the scree plot (vertical line, Fig. 1b).

The mean DI scores of males in these three clusters exhibited considerable range, which reflects the behavioral dynamic and social plasticity in *A. burtoni* communities (Fig. 1a). Importantly, the three clusters do not follow strictly the (−) and (+) DI score dichotomy (Fig. 1a). Namely, the clusters subdivided males with clearly (+) DIs (mean DI 45.44 ± SD 14.09; $n = 7$) and those with clearly (−) DIs (mean DI −21.04 ± SD 9.99; $n = 8$). However, the analysis also suggested an intermediate (INT) cluster of males with DI scores near zero (mean DI 3.78 ± SD 5.17; $n = 7$), based on threat, flee, and shoal behaviors (Fig. 1a).

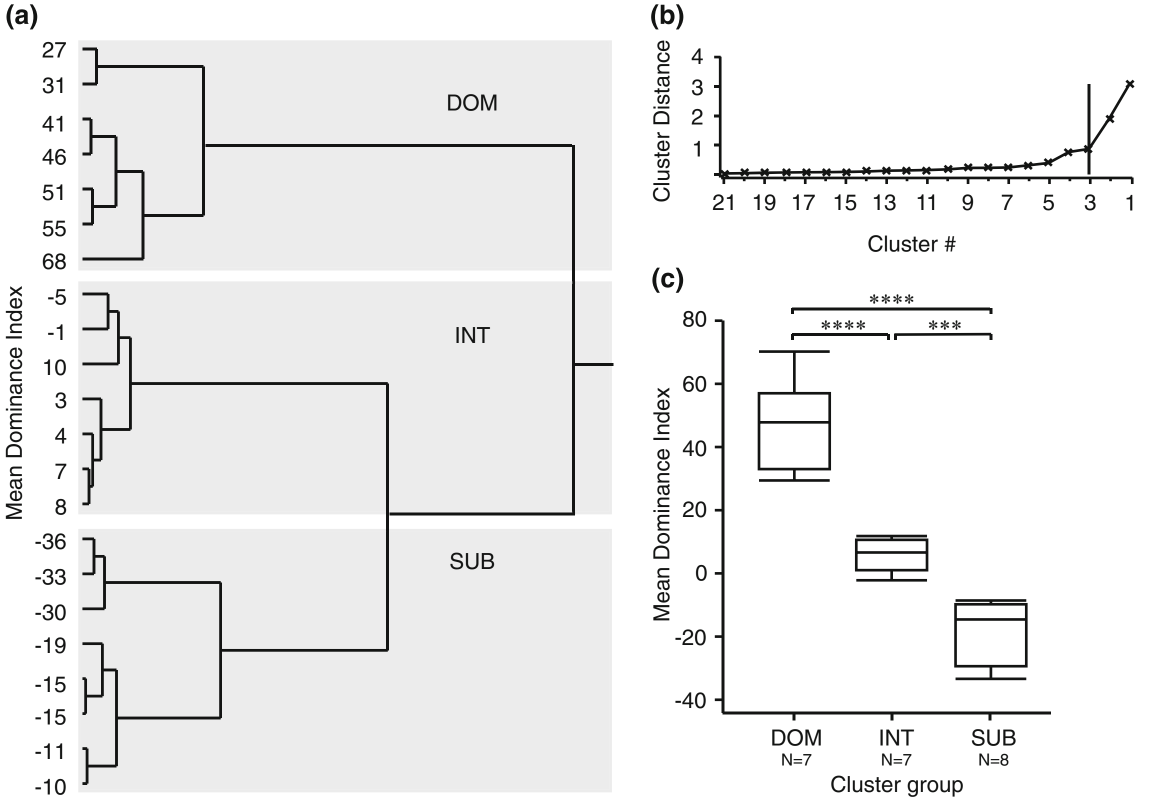

Fig. 1 **a** Distance-scale dendrogram of a hierarchical cluster analysis based on the behavioral variables threat, flee, and shoaling for each male ($n = 22$) using the Ward linkage method. The mean dominance index (DI) score for each of the individual males is indicated (*left axis*). **b** Scree plot showing the distance bridged to join clusters at each step. *Solid vertical line* indicates the parsimonious break point where distance increases abruptly, suggesting three cluster groups. **c** Box plots showing the DI values for dominant (*DOM*), intermediate (*INT*) and submissive and non-territorial (*SUB*) males. The *horizontal line within the box* represents the median sample value. *Brackets* represent post hoc (Tukey) comparisons between the three male cluster groups (**** < 0.0001, *** = 0.0005)

This triadic split was supported by a one-way ANOVA [$F(2,21) = 77.25$, $p < 0.0001$] and subsequent posthoc tests that revealed significant differences between all three clusters (Fig. 1c).

Figure 2 shows a detailed timeline of each male's social trajectory over the entire observation period including the number of social transitions with respect to the DI score. The results highlight the transitional status of males in the INT cluster and the social uncertainty faced by this phenotype (Fig. 2b). This interpretation is supported by the number of zero DI crossover-point transitions made by males in each of the clusters over the course of the observational period (Fig. 2). Six out of seven individuals belonging to the INT cluster made a transition during the observation period. Five of them transitioned more than once (Fig. 2b). In contrast, only one individual belonging to the DOM cluster transitioned, while other DOMs consistently had positive DI scores (Fig. 2a). Similar to the DOMs, only one individual belonging to the SUB cluster made a transition, while other SUBs still had negative DI scores (Fig. 2c).

Together, the results suggest that males with clearly negative DI scores exhibited behaviors and a (subjectively assessed) gross phenotype typical of fully submissive, non-reproductive individuals (prototypical SUB males) and those with clearly positive DI scores exhibited behaviors and a (subjectively assessed) gross phenotype typical of fully territorial and reproductive individuals (prototypical DOM males). However, the phenotype of INT males, i.e., low positive or low negative DI scores, is not readily apparent.

In principle, a DI score close to zero can reflect either the sum of a sizable but similar number of dominant (+) and submissive (−) behaviors, or alternatively, an overall low frequency of social interactions per se. In other words, the DI does not clearly distinguish between active and passive individuals close to the crossover point. In this way, potentially important information about the individual frequency of intrasexual conflict behaviors that regulate phenotypic plasticity in the species may be overlooked.

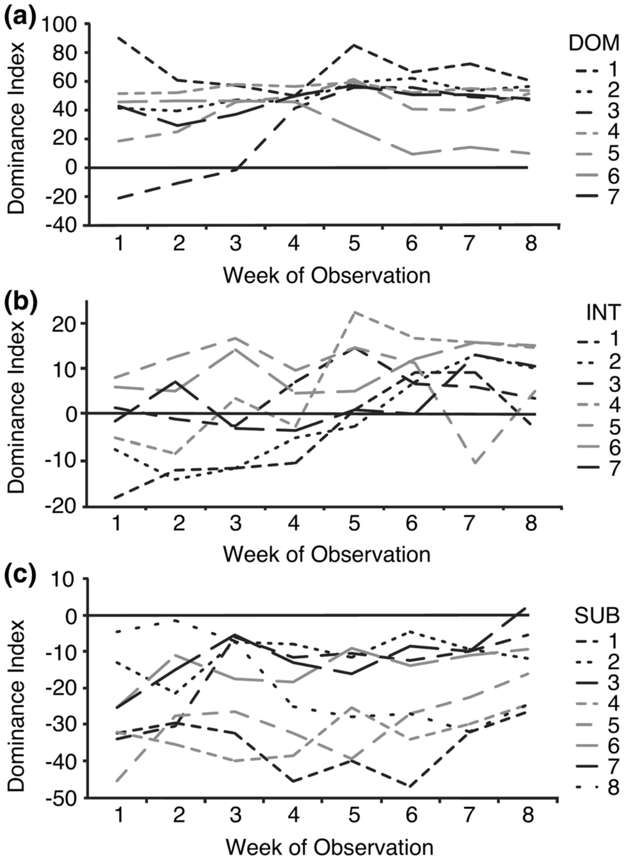

Fig. 2 Timeline plots showing the weekly average DI for each male (*color code* to the *right* for individual identity) belonging to the **a** high DI cluster (*DOM*), **b** the intermediate DI cluster (*INT*), and **c** the low DI cluster (*SUB*). *Dashed lines* indicate the zero DI crossover point for social transitions. For abbreviations, see Fig. 1

To resolve this ambiguity, we developed the CI (see Methods). CI differed significantly within the three clusters [$F(2,21) = 73.76$, $p < 0.0001$; one-way ANOVA]. INT males showed the highest CI (mean 84.23 ± SD 8.34), followed by prototypical DOMs (mean 54.35 ± SD 9.22) and prototypical SUBs (mean 23.42 ± SD 11.03), respectively (Fig. 3).

We next asked if INT cluster males exhibit other behaviors that distinguish them from prototypical SUB and DOM males. To explore the latter notion, we analyzed the frequency of behaviors typically not part of the DI ethogram yet readily observable during agonistic interactions. Indeed, INT males responded to threats either by returning the threat, fleeing, or by failing to be displaced: a behavior named here "ignore threat" (see Table 1; see also Fernald and Hirata 1977). This behavior differed significantly among the clusters [$F(2, 21) = 65.68$, $p < 0.0001$; one-way ANOVA] and, intriguingly, was shown almost exclusively by INT males (Table 2; Fig. 4a). Ignore threat behavior is also positively correlated to CI [$r^2(21) = 0.75$; $p < 0.0001$] (Fig. 4b).

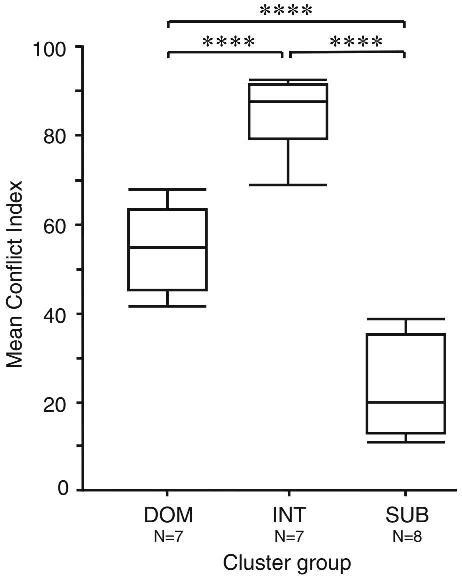

Fig. 3 Differences in conflict index (CI) between cluster groups. *Box plots* showing the distribution of CI values for DOM, INT, and SUB males. The *horizontal line within the box* represents the median sample value. *Brackets* represent post hoc (Tukey) comparisons between the three male groups (**** < 0.0001). For other abbreviations, see Fig. 1

Table 2 Means and SD of male behaviors by phenotype

Behavior	Phenotype	Mean ± SD
All threats	Dominant (DOM)	48.66 ± 9.88
	Intermediate (INT)	39.36 ± 5.09
	Submissive (SUB)	1.09 ± 0.78
Ignore threat	DOM	1.88 ± 2.31
	INT	9.25 ± 1.49
	SUB	0.49 ± 0.33
Flee	DOM	3.80 ± 6.52
	INT	35.61 ± 3.80
	SUB	21.83 ± 10.49
Shoal	DOM	8.97 ± 15.40
	INT	75.44 ± 4.38
	SUB	96.75 ± 2.38
Lachrymal stripe display	DOM	94.46 ± 11.03
	INT	50 ± 5.25
	SUB	3.16 ± 4.15
Vertical stripe display	DOM	39.95 ± 13.78
	INT	73.61 ± 12.52
	SUB	21.48 ± 24.95

The latter result suggests that males that ignore threats are highly active. Our results, however, show that these males are not the ones that also deliver threats most frequently (Fig. 4c). Indeed, the results imply that prototypical DOMs are most frequently threatening, with INT males

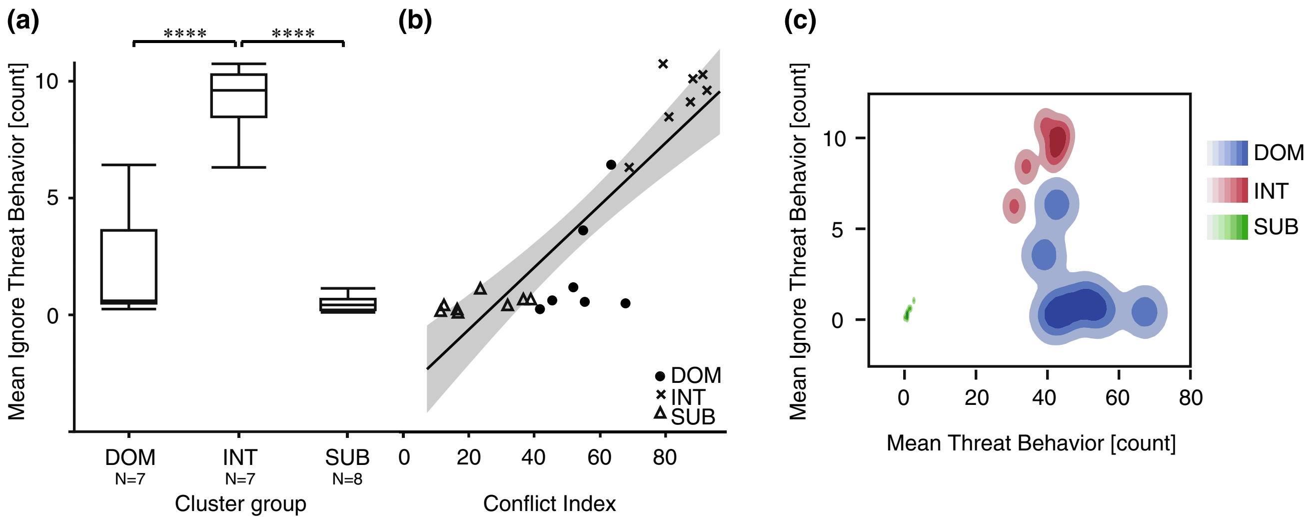

Fig. 4 Differences in frequency of the agonistic behavior "ignore threat" between the three male mean cluster groups. **a** *Box plots* showing the distribution of ignore threat for DOM, INT and SUB males. The *horizontal line within the box* represents the median sample value. *Brackets* represent post hoc (Tukey) comparisons between the three groups (**** ≤ 0.0001). **b** Linear regression between individual CI scores and ignore threat behavior for all males in the three groups ($p < 0.0001$; $n = 22$; $r^2 = 0.75$). *Shaded area* indicates 95% confidence limits. **c** Heat map of the mean total "threat" by the mean total ignore threat per individual indicating the three male cluster groups. For abbreviations, see Figs. 1 and 3

exhibiting threat at frequencies similar to (but slightly lower than) DOMs, and SUBs exhibiting threat with close to zero frequency (Table 2; Fig. 4c). No consistent temporal pattern in the display of this behavior was found in the observations presented here.

Phenotypic pigment displays

Lateral body pigment displays are used by *A. burtoni* males for social signaling (e.g., Baerends and Baerends-Van Roon 1950; Desjardins et al. 2012) and thus might also provide a marker for identifying INT males. The lachrymal stripe display is associated with territorial dominance in *A. burtoni* (Fernald and Hirata 1979; Desjardins et al. 2012). Accordingly, the results showed that the frequency of this display significantly differed within the clusters [$F(2, 21) = 291.43$, $p < 0.0001$; one-way ANOVA]. Prototypical DOMs displayed lachrymal stripes with greatest frequency, followed by INT males and prototypical SUBs, respectively (Table 2; Fig. 5a). A weak yet significant correlation existed between lachrymal stripe display and CI [$r^2(21) = 0.26$; $p = 0.01$] (Fig. 5b).

Another socially mediated reversible pigment display, the vertical stripe display, appears during male–male conflicts and is typically quantified as percent display time during focal observation (Fernald and Hirata 1979) (Table 1). Comparing the frequency of vertical stripe display revealed significant differences among the DI cluster cohorts [$F(2, 21) = 15.20$, $p < 0.0001$], which was driven by a high frequency of occurrence in INT males (Table 2; Fig. 6a). The data also yielded a significant positive correlation between vertical stripe display and CI [$r^2(21) = 0.70$; $p < 0.0001$] (Fig. 6b).

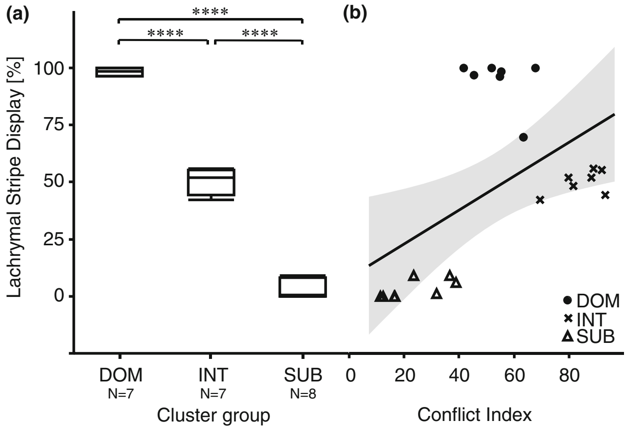

Fig. 5 Differences of lachrymal stripe display between male groups. **a** *Box plots* showing the distribution for lachrymal stripe display (% time) for DOM, INT and SUB males. The *horizontal line within the box* represents the median sample value. *Brackets* represent post hoc (Tukey) comparisons between the three male groups (**** ≤ 0.0001). **b** Linear regression between individual CI scores and lachrymal stripe display for all males in the three groups ($p < 0.0138$; $n = 22$; $r^2 = 0.26$). *Shaded area* indicates 95% confidence limits. For abbreviations, see Figs. 1 and 3

Cluster analysis summary

Finally, to further substantiate and validate the division of males into three groups we expanded our original cluster analysis to include the behavioral variables discussed

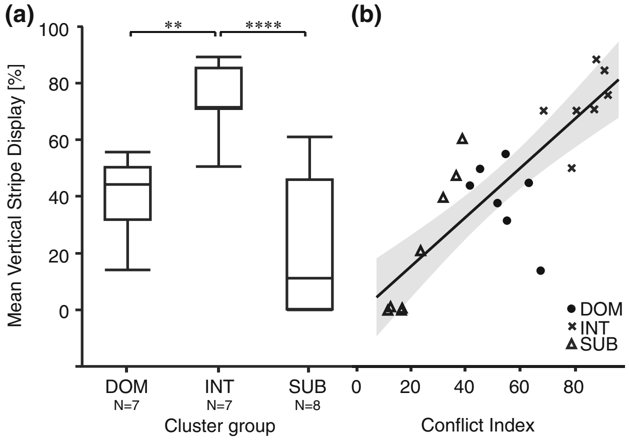

Fig. 6 Differences of vertical stripe display between the three male groups. **a** *Box plots* showing the distribution for stripe display (% time) for DOM, INT and SUB males. The *horizontal line within the box* represents the median sample value. *Brackets* represent post hoc (Tukey) comparisons between the three male groups (**** ≤ 0.0001, ** $= 0.007$). **b** Linear regression between individual CI scores and vertical stripe display for all males in the three groups ($p < 0.0001$; $n = 2$; $r^2 = 0.70$). *Shaded area* indicates 95% confidence limits. For abbreviations, see Figs. 1 and 3

above (i.e., an analysis based on threat, flee, shoal, ignore threat, lachrymal stripe display, and vertical stripe display). Figure 7 shows the clustering sequence and distance scree plot of the 22 males indicating that intracluster homogeneity and intercluster heterogeneity are maximized by a three-cluster solution. Importantly, all individual males remain in the original cluster into which they were sorted previously (see Fig. 2 for fish IDs).

Discussion

The goal of this study was to develop a non-invasive method to further categorize *A. burtoni* males. Specifically, we focused on males that are close to the crossover point between SUB and DOM status as defined by the traditional DI. These INT males showed the highest frequency of intrasexual conflict behaviors, i.e., the highest CI scores. In addition, at least two distinct male–male conflict-related characteristics, the ignore threat behavior and expression of the vertical stripe display, further distinguish these INT males from prototypical SUBs and DOMs. The results suggest that a combined analysis of an extended DI ethogram and CI scores can reliably identify a unique behavioral suite for a distinct group of males in *A. burtoni*.

Our analysis using the traditional DI ethogram, along with an alternative quantification of intrasexual conflict behavior, permits a more nuanced classification of the INT males as a distinct phenotype. These analyses contribute to a better understanding of the relationships between social status and risk-avoiding and/or aggression-reducing strategies that are currently being studied in many taxa (e.g., Judge and De Waal 1993; Harris et al. 2010; Černá et al. 2013). The finding that males in more variable social circumstances engage more frequently in a wider range of social behaviors provides a new avenue for research on facultative change in competitive strategy based on fluctuating conditions in the social environment. This adaptive link is also the subject of attention in multiple taxa and at multiple levels of analysis (e.g., Wiebe 1995; Mautz and Jennions 2011). Such tactics are of particular interest in phenotypically plastic organisms due to their overt influence on individual morphology, which in turn influences the morphology of other individuals (e.g., Furness et al. 2015). Rapid, reversible changes in individual morphology, physiology, and behavior affect the hierarchical arrangement of communities (Whitham et al. 2003; Smith et al. 2015). *A. burtoni* is useful as a model organism in this

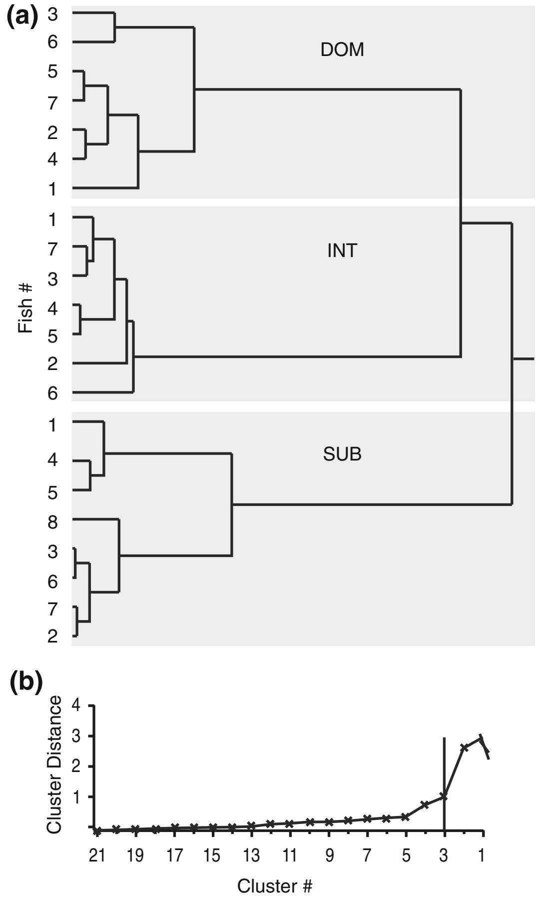

Fig. 7 **a** Distance-scale dendrogram of a hierarchical cluster analysis of all behavior variables described in this study (threat, flee, shoal, ignore threat, lachrymal stripe display, and vertical stripe display) for each individual male ($n = 22$) using the Ward linkage method. **b** Scree plot showing the distance bridged to join clusters at each step. *Solid line* indicates the parsimonious break point where distance increases abruptly, suggesting three clusters

burgeoning area of study due to the complex and conspicuous influence of individual male behavior and morphology on other individuals, particularly in regard to reproduction (e.g., Clement et al. 2005; Oliveira et al. 2005; Desjardins et al. 2012).

Although not specifically tested here, it is conceivable that INT males can be identified as those transitioning to or from DOM/territorial/reproductive status. Such individuals have been described previously as expressing physiological changes associated with behavioral changes during social ascent and descent (e.g., Hofmann et al. 1999; Burmeister et al. 2005; Parikh et al. 2006; Maruska and Fernald 2010; Maruska et al. 2012). Bi-directional transitioning between SUB and DOM status depends on environmental and social context (Hofmann et al. 1999; Hofmann et al. 2001; Clement et al. 2005; Fernald 2007; Desjardins and Fernald 2008; Korzan et al. 2008) as well as on growth rate (Hofmann et al. 1999). Moreover, transitional males exhibit increased intrasexual aggression, and body pigment more similar to DOMs within hours of increased intrasexual victory and correspondingly increased androgen circulation (Parikh et al. 2006). Thus, the high CI scores of INT males described here may illuminate the behavioral mechanisms and costs during the transition from SUB to DOM status.

Why do INT males ignore threats?

Our data show that males with DI scores near zero engage more frequently in intrasexual conflict (having the highest CI scores), and engage in a wider variety of conflict behaviors, than either prototypical SUB or DOM males. This trait is consistent with the necessity to adjust most rapidly to unstable social circumstances. The transition from SUB to DOM status requires vigilant testing and/or observation of DOM males (Desjardins et al. 2012). Therefore, these males must have a mechanism for defraying costs of aggression from DOMs.

These unique challenges may explain the higher frequency of the ignore threat behavior in INT males relative to prototypical SUB and DOM males. Ignoring threats may be a strategy for avoiding risk, testing competitors or competitive events without risk to status (e.g., Judge and De Waal 1993; Grosenick et al. 2007). A male ignoring a threat avoids both escalating a contest and the conspicuous social defeat of fleeing behavior. This interpretation is supported in Fig. 4c, where we demonstrate that INT males ignoring threats most frequently do not deliver threats most frequently. As such, these males are likely better equipped to handle confrontation than prototypical SUBs and may ignore threats to better assess the relative threat level of an opponent. Partly due to bright coloration and larger size, males transitioning to or from DOM status are not likely to avoid attention from threatening individuals as effectively as prototypical SUBs (Greenwood and Fernald 2004). Males transitioning between social states (near-zero DI) may benefit from more frequent tests of rivals and therefore engage in more confrontations than either prototypical, more submissive SUBs or prototypical, more established DOMs. Threats by rivals may not be successful, in which case energy, social spacing, and status (along with the corresponding hormonal suite) may be conserved by not engaging in stress-based escape or escalated aggression. Interestingly, Fernald and Hirata (1977) describe a behavior comparable to (or the same as) ignore threat, where males in the process of establishing a territory may fail to flee or fight when attacked.

By definition SUB males have the lowest ratio of wins to losses in intrasexual conflict. A prototypical SUB remaining in place (or ignoring a threat) during a confrontation may be at greater risk of attack and defeat than any other male doing the same. Additionally, DOM males risk the loss of acquired territory/status in a given confrontation (e.g., their reproductive status), and a passive response may be much costlier to this group than to any other. In contrast, INT males have higher win/loss ratios, but no territories to defend. In these ways they are uniquely situated as being more capable of winning confrontations than prototypical SUBs, with less to lose in defeat than prototypical DOMs.

Facultative behavioral response to competitor rank has already been demonstrated in *A. burtoni* males, consistent with our hypothesis regarding the ignore threat behavior. An attention hierarchy has been demonstrated in this species, where individuals observe competitive third party interactions and modify their behavior accordingly (Grosenick et al. 2007). Individuals may hasten social ascent and maximize the window of reproductive competence by picking and choosing their fights based on these observations of other males (Desjardins et al. 2012). The range of behaviors involved in this picking and choosing may be expanded by our findings and their expansion on the behavior observed in Fernald and Hirata (1977). Males approaching DOM status have different opportunities/capacities for reproduction (Fernald 2007; Renn et al. 2009; Kustan et al. 2011). It is likely that the threat presented by competitors will vary correspondingly (Hofmann et al. 1999; Maher and Lott 2000; Greenwood and Fernald 2004), and so a behavioral mechanism for varied response should exist.

How do INT males use pigment displays?

Lachrymal stripe display

The lachrymal stripe is associated with territorial acquisition and agonistic behavior (Heiligenberg et al. 1972; Desjardins and Fernald 2008; Desjardins et al. 2012), suggesting that a dark lachrymal stripe may be an honest signal (Zahavi 1993) of conflict ability. Our data support that association in showing that the display is most frequent in prototypical DOMs. These males would be most likely to consistently win confrontations (e.g., Hofmann 1999; Dugatkin and Druen 2004; Dugatkin and Earley 2004; Oliveira et al. 2002, 2005) thereby increasing tenure as DOMs. However, INT males may also display lachrymal stripes clearly distinguishing them from the prototypical SUBs.

INT males engage in more confrontations, with less certain outcomes, than other males, and so may benefit from activating or deactivating the signal based on a rival's relative competitive status. Intrasexual confrontations which are evenly matched or in which a male is victorious increase androgen expression, and logically will increase expression of the androgen-correlated lachrymal stripe (Muske and Fernald 1987). *Oreochromis mossambicus*, another cichlid species, has been shown to increase androgen expression during display against a mirror reflection (an evenly matched fight) and to experience a "winner effect" similar to that of fish victorious in intraspecific combat (Oliveira et al. 2005; Dijkstra et al. 2012).

Vertical stripe display

The vertical stripe is a conspicuous visual signal, which can be darkened and lightened from moment to moment (Fernald and Hirata 1977, 1979). Vertical stripes cover a large area on the lateral body (Fernald and Hirata 1979) and may be displayed as a signal of social defeat, conspicuous to an attacking male. *A. burtoni* males are believed to visually assess the relative strength of competitors (Desjardins et al. 2012). By exhibiting the vertical stripes as a submissive signal, defeated males might curtail further agonism (Desjardins and Fernald 2008). Thus the fact that INT males show the highest number of agonistic interactions (i.e., high CI), but also exhibit vertical stripes more frequently than other males might, suggests that this display provides a tactic for avoiding costly attacks by submission (Fig. 6a, b).

Variations between SUB and DOM phenotypes in dimensions of reproduction, stress response and social ecology have been described (Ferno 1987; Fox et al. 1997; White et al. 2002; Clement et al. 2005; Parikh et al. 2006; Korzan et al. 2008). It is also important to consider nonlinear variation in these traits during transition, as suggested by the findings of Fernald and Hirata (1977) where wild *A. burtoni* males exhibited unique responses to aggression while establishing territory.

As such, INTs may represent males transitioning in territorial status (Hofmann et al. 1999). These males do not only occupy a central place on the spectrum of some traits expressed in nonterritorial and territorial males, but also exhibit some traits with greater frequency than both prototypical SUB and DOM males. Indeed, tactics used by these transitioning or uncertain individuals may not match with the needs and traits of more subordinate or more dominant individuals, as manifest in traditional hierarchical classifications (e.g., Drews 1993), particularly as pertains to behavioral and social plasticity. The heightened behavioral plasticity in a competitive context of individuals experiencing greater fluctuation in social circumstance may demonstrate the adaptive value of facultative strategies under such conditions; (e.g., Wiebe 1995; Mautz and Jennions 2011) particularly as pertains to the energy expended on territorial maintenance behaviors (e.g., Lederer 1981). Phenotypically reversible organisms exhibit direct and cyclical links between and among behavioral variation, morphology, and the composition of social groups. These uniquely conspicuous connections provide a valuable avenue for analyzing the way in which short-term intraindividual and intrapopulation changes in social behavior influence long-term behavioral, ecological, physiological, and epigenetic traits (e.g., Oliveira 2012).

In conclusion, we show that modified ethograms for male *A. burtoni* allow for classification of additional behavioral phenotypes beyond a strict SUB-DOM dichotomy, more fully capturing the complex social dynamic of this model organism. More broadly, using cluster analysis based on intrasexual conflict behaviors and facultative competitor-testing or risk-avoiding strategies might allow for a more nuanced understanding of social hierarchies in many species.

Acknowledgements The authors are grateful to M. E. Hauber for his suggestions, H. Hofmann for valuable comments and discussion, Z. Baranov for valuable input, members of the Preuss lab for maintaining fish, and for funding provided by the following sources: a National Science Foundation Graduate Research Fellowship issued to A. Fulmer (No. 2012143588), and National Science Foundation grants IOS 0946637 and IOS 11471172 issued to T. Preuss. Additionally, we are grateful to earlier reviewers for helpful comments.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The regulations of the Hunter College CUNY IACUC were followed at all stages of this study.

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