CHAPTER

4.3

Alternative Reproductive Tactics in Blennies

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INTRODUCTION

WHAT ARE ALTERNATIVE REPRODUCTIVE TACTICS?

In some species, individuals of the same sex adopt alternative tactics to compete for mates. Instead of basing their intra-sexual competition in the investment of resources towards the attraction and monopolisation of sex partners (e.g. expression of visual, chemical or acoustic courtship signals and/or the defense of breeding territories), individuals using the alternative tactic exploit the investment made by conventional individuals (e.g., by sneaking into the breeding territories during spawning episodes). Therefore, the terms bourgeois and parasitic have been proposed to describe these two tactics (Taborsky, 1997) and the traits selected in the two male types are usually divergent. In bourgeois males,

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traits related with mate attraction and monopolisation will be selected, while in parasitic males those traits that increase the probability of stealing fertilisations from bourgeois males will prevail. This disruptive selection, acting on a constellation of phenotypic traits, may result in the evolution of polyphenisms within one sex, usually the males, where the expression of male reproductive behaviour and male secondary sex characters in parasitic males becomes dissociated from the differentiation of a functional male gonad. In summary, alternative reproductive tactics (ART) are discontinuous behavioural and morphological traits selected to maximise fitness in two or more mutually exclusive ways in the context of reproductive competition. Based on the descriptive patterns of observed behaviour, alternative reproductive phenotypes can be categorised as fixed or plastic (Moore, 1991; Brockmann, 2001). In fixed alternative phenotypes, the individuals adopt one of the tactics for their entire lifetime. In plastic (or flexible) alternative phenotypes, the individuals may change tactics during their lifetime. Within plastic ART, again, two sub-categories can be further distinguished: irreversible sequential patterns, when individuals switch from one tactic to another at a particular moment in their lifetime (developmental switches); and reversible patterns, when individuals can change back and forth between patterns (Moore, 1991; Moore *et al.*, 1998; Brockmann, 2001; Fig. 4.3.1).

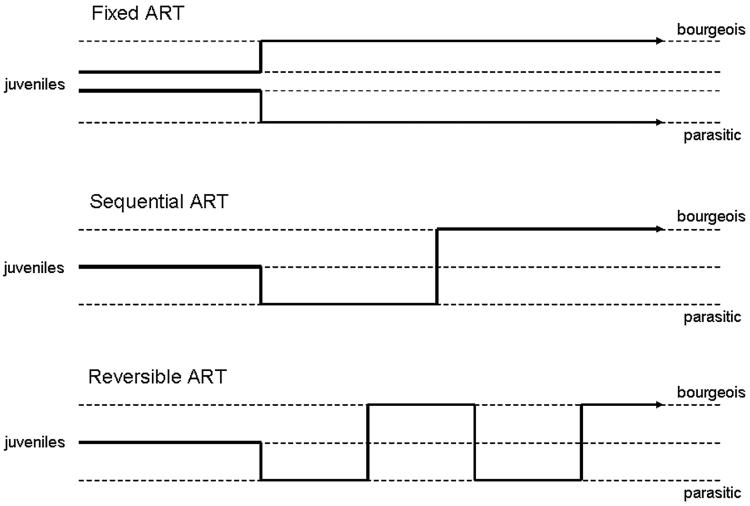

Fig. 4.3.1 In fixed ART, animals adopt one of the tactics for their lifetime. In plastic ART, animals may irreversibly switch from one tactic to the other (sequential tactics) or switch back and forth between tactics (reversible tactics).

Other schemes of classification of alternative reproductive phenotypes have been proposed in the literature based on the underlying evolutionary processes such as isogignous (i.e., with equal fitness outcomes) versus allogignous (i.e., not equally successful strategies, Austad, 1984), alternative versus conditional strategies (Gross, 1996), Mendelian strategies versus developmental strategies versus behavioural strategies (Shuster and Wade, 2003). However, these approaches require a great deal of information to be collected before the alternative phenotypes can be classified. For example, the classic distinction between frequency-dependent and condition-dependent strategies requires the knowledge about the lifetime reproductive success of the alternative phenotypes, since in the former they are expected to be isogignous while in the latter this is not expected (in this case, alternative phenotypes were seen ~~h~~as 'making the best of a bad job', Dawkins, 1980). Moreover, the simple dichotomy between alternative versus conditional strategies requires the investigation of the genetic mechanisms underlying the sexual polymorphism in the population (see below). Therefore, we have adopted a descriptive classification of ART.

ORIGIN OF ALTERNATIVE REPRODUCTIVE TACTICS: EVOLUTIONARY AND DEVELOPMENTAL MECHANISMS

Alternative reproductive tactics do not necessarily imply the occurrence of genetic polymorphisms. They may emerge both from monomorphic and polymorphic genotypes (Austad, 1984; Gross, 1996; Shuster and Wade, 2003). When there is no genetic variation underlying the expression of alternative phenotypes (genetic monomorphism), these may be conditionally triggered by current conditions or by developmental switches. Therefore, alternative tactics emerge among individuals with the same genetic architecture due to the diverging conditions that they experience. Usually, thresholds exist that determine the switching point between conditional tactics. For example, the decision to adopt one or the other tactic may depend on a size threshold so that above a certain size, individuals would do better by continuing to grow and postponing reproduction (as a bourgeois male), while below the threshold it may pay to reproduce early as a parasitic breeder (i.e., condition-dependent selection, Fig. 4.3.2a; Taborsky *et al.*, 2008). Alternative reproductive tactics may also result from polymorphic genotypes, in which case the frequencies of the each tactic-based genotype should be balanced by

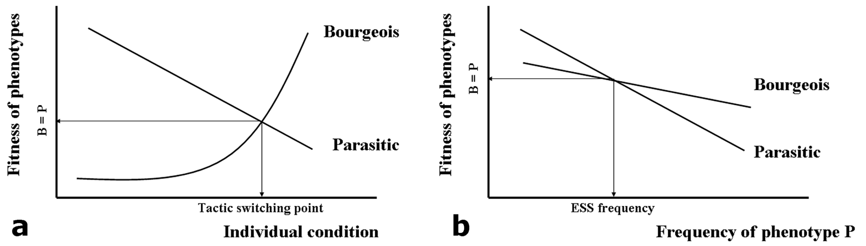

Fig. 4.3.2 **a.** Condition-dependent ART are based on genetic monomorphism, where the individual condition determines the best tactic to adopt. For example, males may gain by adopting a parasitic tactic while they are small and switch into a bourgeois tactic when they grow. **b.** Frequency-dependent ART are based on polymorphic genotypes. In this case, the lifetime fitness of animals using the different tactics is expected to be equal and the frequencies of each tactic-based genotype are balanced by frequency-dependent selection.

frequency dependent selection (Fig. 4.3.2b), leading to equal lifetime fitness of animals using the different tactics (Shuster and Wade, 1991; Ryan *et al.*, 1992; Repka and Gross, 1995). There has been considerable debate in the existing literature on the relative importance of conditional tactics as opposed to genetic polymorphism for the evolution of alternative reproductive tactics (Gross, 1996; Shuster and Wade, 2003). Since conditional responses have been demonstrated for the majority of the cases of alternative reproductive tactics described so far and since conditional choices have been associated with genetic monomorphism, it has been argued that genetic polymorphisms play a minor role in the causation of alternative tactics (Gross, 1996; but see also Shuster and Wade, 2003). This dichotomy has led to a terminological distinction often made in evolutionary biology where the term strategy is used to classify a 'genetically based program', while the tactic describes the rules that are part of a strategy (i.e., the phenotype; Shuster and Wade, 2003). Despite this ongoing debate, it is too reductionist to assume that alternative tactics could origin purely from either genetic or environmental factors (Caro and Bateson, 1986). In fact, most dimorphic traits seem to be threshold traits influenced by quantitative trait loci, which means that tactic switch points can have a genetic basis, implying that trait expression is both conditional and heritable (Tomkins *et al.*, 2004). This scenario allows alternative phenotypes to evolve independently from each other (i.e., in a non-frequency dependent fashion), which greatly increases the scope for the evolution of alternative tactics (Taborsky *et al.*, 2008). In this chapter, we have opted to use the term 'tactic' because we will mainly deal with phenotypes and not with the functional implications of the term 'strategy'.

PHYSIOLOGICAL MECHANISM UNDERLYING THE EXPRESSION OF ALTERNATIVE REPRODUCTIVE TACTICS

Due to their role in sexual differentiation and in the control of male reproduction in vertebrates (e.g., Dixon, 1998; Wilson *et al.*, 2002; Nelson, 2005), sex steroids are prime candidates to play a key role in the causal mechanisms underlying the differentiation and the maintenance of alternative reproductive morphs. In particular, androgens participate in the differentiation of primary and secondary sex characters, in the expression of reproductive behaviours, in the feedback regulation of the hypothalamus and pituitary, and in spermatogenesis (Nelson, 2005), which makes them a preferential target for studies of endocrine correlates of male ARTs. A review of the available data on androgen levels in species with ART indicates that alternative phenotypes in teleost species consistently differ in their circulating 11-ketotestosterone (KT) levels, with bourgeois males having significantly higher levels of circulating KT than parasitic males, but that there was no clear pattern regarding testosterone (T) (Brantley *et al.*, 1993a; Oliveira, 2006). Interestingly, the exceptions to this rule occur in species where the ART do not involve morphological specialisations (i.e., polyphenisms), suggesting that androgens are particularly relevant for the expression of tactic-dependent morphological traits but not as much for the expression of tactic-specific behavioural traits (Oliveira, 2006). Therefore, the development of male ARTs is likely to be influenced by other neuroendocrine systems in addition to gonadal steroids.

Another neuroendocrine candidate that has received growing attention in recent years is the neuropeptide arginine vasotocin (AVT). This decapeptide is the teleost homologue to the mammalian arginine vasopressin (AVP) and, together, AVP/AVT have been demonstrated to influence the expression of social behaviours—including courtship behaviour—in a wide range of vertebrate species (Goodson and Bass, 2001). In all the teleost species with ART studied so far, an association has been found between the expression of alternative phenotypes and forebrain AVT, either in terms of soma size or number of AVT-ir neurons, or of AVT mRNA expression on a per cell basis (see Oliveira, 2006 for a review). Furthermore, in studies that have manipulated AVT by exogenous administration in fish species with ART, AVT has been effective in eliciting tactic-specific behaviours (Goodson and Bass, 2000; Carneiro *et al.*, 2003; Semsar and Godwin, 2003).

Finally, glucocorticoids have also been suggested as potential players in ART differentiation (Moore *et al.*, 1998). Recent studies in fish suggest that glucocorticoid profiles are associated with distinctive behavioural styles and that the interactions between the hypothalamic-pituitary-interrenal axi~~a~~s and the hypotalamic-pituitary-gonadal axis could be the causal link to explain the glucocorticoid modulation of alternative reproductive traits (Knapp, 2004).

To summarise, in recent years, we have moved from a simplistic view of the causal mechanisms of ART being based in pleiotropic-like effects of one endocrine agent on a constellation of tactic-specific traits, to a more complex model in which different neuroendocrine mechanisms interact in the control of the different traits that together make-up the specific profiles of alternative phenotypes (Fig. 4.3.3).

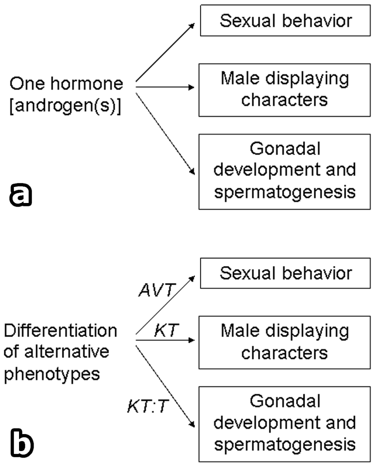

Fig. 4.3.3 **a.** Classically, the causal mechanisms of ART were explained by one endocrine agent acting on all tactic-specific traits. For example, androgens have been suggested to be causally linked to the behavioural, morphological, anatomical and gonadal traits of bourgeois males. **b.** Recently, the interaction between different neuroendocrine mechanisms has been suggested to be necessary to induce and maintain alternative phenotypes.

OCCURRENCE OF ALTERNATIVE REPRODUCTIVE TACTICS IN BLENNIIDS

Teleosts are by far the vertebrate taxon with the highest incidence of species with ART (for comparative data on ART in different vertebrate taxa see Oliveira *et al.*, 2008). The last published count of ART in fish identified 170 species from 32 different families (Taborsky, 2008), and new examples of species with ART are described annually, even among common temperate species studied on a regular basis. Three main factors have been suggested to explain this ubiquity of ART among teleosts: (1) the occurrence of indeterminate growth in fish that promotes large intra-sexual size differences; (2) external fertilisation; and (3) male parental care (Taborsky, 1999).

Among blennioids, ART have been described in 10 species from the families Blenniidae and Tripterygidae (Table 4.3.1). No published records that we are aware of have described ART in the other 4 Blennioid families (Chaenopsidae, Clinidae, Dactyloscopidae and Labrisomidae). A phylogenetic analysis of ART in blenniids suggests that this trait evolved several times and that it never become a permanent feature of a deeper clade, comprising many genera (Almada and Robalo, 2008). Two Northeastern Atlantic blenniids have been studied in great detail regarding both the mechanisms and the ecology of ART and, therefore, we will concentrate on these two species in the following section.

Table 4.3.1 Blenniioid species with alternative reproductive tactics

Family	*Species*	*References*
Tripterygidae	*Axoclinus nigricaudus*	Neat (2001)
	Axoclinus carminalis	Thresher (1984); Neat (2001)
	Tripterygion tripteronotus	Wirtz (1978); Mohr (1986); de Jonge and Videler (1989)
	Tripterygion delaisi	Wirtz (1978); Mohr (1986); de Jonge and Videler (1989)
	Tripterygion melanurus	Mohr (1986)
Blenniidae	*Ophioblennius atlanticus*	N. Bouton and R. S. Santos, pers. com.
	Scartella cristata	Neat *et al.* (2003a); MacKiewicz *et al.* (2005)
	Parablennius parvicornis	Santos (1985b); Oliveira *et al.* (2001b)
	Salaria pavo	Ruchon *et al.* (1995); Gonçalves *et al.* (1996)
	Salaria fluviatilis	Neat *et al.* (2003b)

TWO TALES FROM THE INTERTIDAL: SATELLITES AND SNEAKERS

‘Satellites of Love’: Satellite Males in the Azorean Rock-pool Blenny

The mating system of the Azorean rock-pool blenny *Parablennius parvicornis* has been well characterised in a population occurring at the south coast of Faial, one of the islands in the central group of the Azores.

In the main studied site, Feteira (38° 31′N; 28° 40′W), some horizontal enclosures in a large basaltic platform have collapsed giving rise to large inter-tidal enclosures that remain with shallow water~~s~~ during low tide (Fig. 4.3.4a). These pools accommodate dense aggregations of *P. parvicornis* and offer perfect conditions for behavioural observations and field experiments (Fig. 4.3.4b, c). Male sequential polymorphism associated with alternative reproductive tactics has been described in this population (Santos, 1985a, b; Oliveira *et al.*, 2001b). These include a male morph without secondary sexual character expression (M–) and involved in parasitic tactics: sneakers and satellites; and a morph characterised by the presence of secondary sexual characters (M+) that may reproduce as nest-holders or act as non-territorial floaters (Santos and Almada, 1988; Fig. 4.3.4d).

At the end of May, males start to compete aggressively for natural cavities in the pools and under boulders (Santos, 1985b). Only the larger males establish territories and many smaller males are seen in the pools and adjacent deeper littoral (Santos *et al.*, 1995; Oliveira *et al.*, 2005). Territorial males change to a distinct brown-dark coloration, and possess well-developed secondary sexual characters, including a distinct head hump and an anal gland in the first two rays of the anal fin (Santos, 1985b). They clean out the crevice or an area under the boulder from sand and debris, and this serves as their nest. Nest-holding males court females by signaling the location of the nest, circling, and leading the females (Santos and Barreiros, 1993). Bigger males are more active in all these behaviours, probably signaling their higher competitive ability and larger resources (Oliveira *et al.*, 2000). The anal glands are likely to play a role in attracting females, as in other blennies they have been shown to release pheromones (see Chapter 5.1 by Barata and Gonçalves, this book). Spawning occurs inside the nest and after laying demersal adhesive eggs the female leaves the nest. Over the reproductive season, the nest-holding males receive spawns of multiple females and their nests may hold more than 30000 eggs (Santos, 1989; Santos and Barreiros, 1993). The male takes care of the eggs until hatching and egg care behaviours include cleaning out the nest of debris, territorial defense against egg predators and replacing water in the nest with a fanning movement of the tail in order to provide the eggs with oxygen.

Although males and females remain in the same pool area for several years, the females are less site-attached than males (Santos and Almada, 1988; Carneiro *et al.*, 2001). Females may be seen in different pools during high tide and often leave again with low tide (Santos and Almada, 1988). During their passage, the nest-holding males actively court females. Some females stay in the pools during low tide and often aggregate in groups. Fecund females overlap in body size with the males (total length = 11 ± 2.0 cm) and between June and July, they lay sequential clutches of eggs (Santos, 1985b; Santos and Almada, 1988). Females show a preference to spawn in the relatively large nests of the bigger males (Oliveira *et al.*, 2000). During the reproductive season, all potential nests are occupied and many aggressive interactions can be observed near the nests (Santos and Barreiros, 1993). Although only the larger males manage to establish and maintain a nest-holding territory, nest defense is difficult, either because the nests are exposed or have a large opening (for example, under boulders), or because the nest has two or more openings that cannot be simultaneously monitored (Santos, 1985a; Oliveira *et al.*, 2000). This allows ample opportunity for smaller sub-dominant males to sneak into the nest and get access to the eggs, allowing male alternative reproductive tactics to evolve. *P. parvicornis* males mature after their first winter when they are about 7 cm in length. These low-competitive males have developed testes and sperm in their *vas deferens* (Santos, 1985b). Testes weight corrected for body weight (gonadosomatic index) is significantly higher in these young males than in the nest-holding males (Santos, 1995; Fig. 4.3.4e). They further differ from the larger M+ males in that they do not express secondary sexual characters (e.g., anal glands, Fig. 4.3.4f; the M+ versus M– morph; Santos, 1985b). The smaller M– males roam around several pools, trying to enter different nests in order to fertilise eggs, and are named 'sneakers'. Larger M– males associate with one or two nests in the same pool and are named 'satellite' males. These males defend the nests they associate with, displaying aggressive behaviours towards nearly all the conspecifics or heterospecifics that enter the territory, with the exception of females and the nest-holder. Satellites are too small to take over and defend a nest, as becomes evident when occasionally a nest-holder disappears due to predation or exhaustion. Initially, the satellite might defend the nest opening against intruders, but soon a larger male will enter the nest and establish himself as a new nest-holder. Santos (1985b) found that associations between a parental male and one or two satellites are often stable over time. These observations have put forward the following question: Why do satellites defend nests? And why do nest-holders allow these fertile males to associate with their nests? To answer these questions, a brief description of the possible costs and benefits associated with each of the tactics is presented.

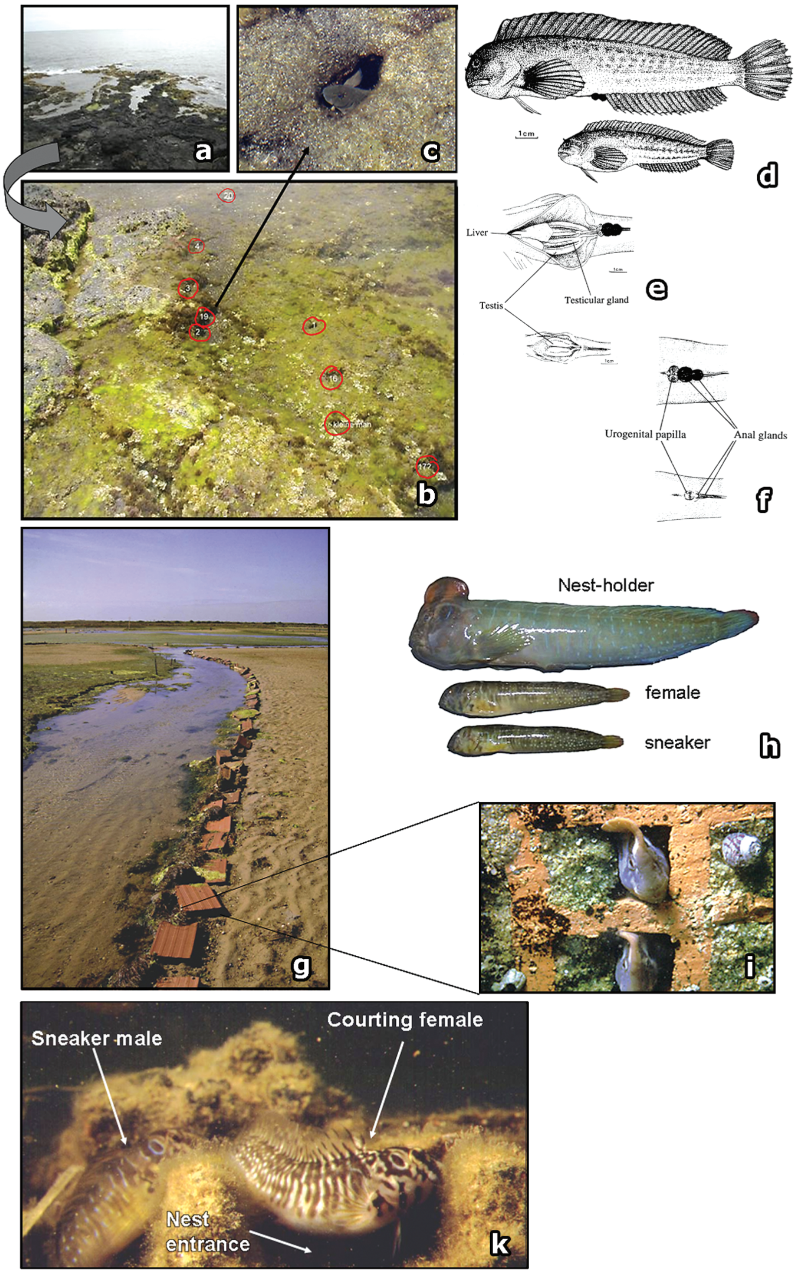

Fig. 4.3.4 **A-J** Alternative reproductive tactics in the rock-pool blenny *P. parvicornis*. **a.** A general view of the intertidal enclosures in Faial (Azores islands) where the studied

Fig. 4.3.4 Contd.

Nest-holding males can be found nearly all the time in or near (within 20 cm) the nest and they respond aggressively towards conspecifics and other species that come close to the nest (Santos, 1985a; Ros *et al.*, 2004). This aggression is highly effective to protect the eggs from predation, as is illustrated by capturing the nest-holder from the nest whereafter many fish home in and remove the eggs within minutes (Santos and Almada, 1988). The high investment of nest-holder males in territorial defense and nest care is costly not only in terms of the allocation of metabolic resources but also because this double investment impedes the male from leaving the nest to forage (Santos and Almada, 1988; Ros *et al.*, 2004). This results in a substantial decrease in body condition over the reproductive period (up to 30% of bodyweight, Santos *et al.*, 1996). Nest-holders need to trade-off vigilance and territorial activities with egg care and mating behaviour. This, together with the fact that many nests have a complex morphology, leaves many opportunities for small males to sneak fertilisations. Larger M– males might have developed the territorial satellite tactic in order to monopolise these sneaking opportunities. Satellites may build up knowledge about the properties of the nest and its surroundings increasing their success in escaping the aggressive advances of the nest-holder and in detecting the best opportunities for parasitic fertilisations of the eggs.

Satellites have been reported to be much more successful to enter the nest during the mating phase of the parental males (observations up to 60 sec) than non-attached sneakers (Santos, 1985b). Nest-holders, thus, loose part of their reproductive potential to the satellites, and respond accordingly. Fifty percent of their aggressive behaviour is directed to the satellite (Santos, 1985b). Still, if corrected for the time present on the

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Fig. 4.3.4 Contd.

population occurs. **b.** A detailed view of one intertidal enclosure. The nests of nine bourgeois males are marked with a red circle. **c.** A bourgeois male at its nest entrance. **d.** Alternative reproductive phenotypes include a larger male morph with secondary sexual characters (M+) that may reproduce as nest-holders or act as non-territorial floaters and a smaller male morph without secondary sexual character expression (M-) involved in sneaker and satellite tactics. **e.** When compared with M+ males (top image), M- males have larger testis and lack a testicular gland (bottom image). **f.** M+ males have an anal gland in the first two rays of the anal fin (top image) that M- males lack (bottom image); (photo credits: a, Rui Oliveira, b and c, Albert Ros; drawings d, e and f adapted from Santos, 1995). **g.** General view of the habitat of the peacock blenny *S. pavo* at the Ria Formosa (southern Portugal). **h.** A female-like sneaker male, a female and a nesting male of *S. pavo*. **i.** Two nesting males in adjacent holes of a brick (photos g and i by Rui Oliveira; photo h by David Gonçalves). **k.** A courting female is trying to enter a nest of a bourgeois male while a female-like sneaker male is in close proximity (photo by Rui Oliveira).

nest-holder territory and compared to other conspecifics, it becomes clear that the nest-holders direct proportionally less aggressive acts towards satellites (Oliveira *et al.*, 2002). This suggests that nest-holders benefit from the presence of satellites, being nest-holders and satellites involved in a reciprocal altruistic relationship (Santos and Almada, 1988). The possible beneficial effect of satellites for nest-holders has been investigated by comparing nests with and without an associated satellite male (Oliveira *et al.*, 2002). Satellites have attacked or chased sneakers and other conspecifics that might compete for fertilisations in the nest (neighboring males and floaters) (see also Santos, 1985b). This shared territoriality was negatively correlated with the time nest-holders invested per challenge on their territory (Oliveira *et al.*, 2002). Moreover, satellites never attack females and have even been seen to court females (loop swimming; Santos, 1985b), which may increase female attraction and also benefit the nest-holder. Satellite removal and replacement experiments have not been carried out yet but are critical in showing whether satellites really accrue benefits in terms of shared territoriality and attracting females. In agreement with the hypothesis that satellites trade-off this investment by exploring the reproductive potential of males, satellites preferred bigger nests and nests with two openings over smaller nests, and were found to associate more with more successful nest-holders. However, paternity data demonstrating that satellites gain an advantage in parasitic fertilisations of the eggs when compared with other parasitic males is still lacking.

From the female perspective, it might be beneficial to select a male associated with a satellite because they signal high quality males, and/or because the territorial behaviour of the satellite reduces the amount of sneaking attempts which might interrupt the spawning bout (discussed by Oliveira *et al.*, 2002). Furthermore, females might benefit from sperm diversity, which increases the genetic variability of the offspring. It is, thus, conceivable that a trade-off could have evolved, in which parental males have to pay some costs in lost fertilisations compensated for by lower costs in nest defense and possibly higher attractiveness (Santos and Almada, 1988).

Change in polymorphism in the Azorean rock-pool blenny is sequential (Santos, 1995; Oliveira *et al.*, 2001b). Males of one to two years of age show the M– morph and may switch between sneaker and satellite tactics. Males older than two years develop secondary sexual characters and compete for nests to become nest-holders or are non-territorial floaters. The morphological changes associated with the switch from M– to M+ males seem to be mediated by androgens, specifically by KT. During the reproductive season, KT and T are elevated in M+ males, while in M– males only T is elevated (Oliveira *et al.*, 2001b; Ros *et al.*, 2006b). Moreover, free circulating levels of KT but not T show a positive correlation with the size of the primary secondary sexual character, the anal glands (Oliveira *et al.*, 2001b), and treatment of M- males with slow release implants of KT but not T induces the growth of these glands (Oliveira *et al.*, 2001c; Ros *et al.*, 2006a).

The relationship between androgen levels and behavioural tactics in *P. parvicornis* seems less straightforward than the just described relation between androgens and morphotype (Oliveira *et al.*, 2001b). Despite the conspicuous within-morphotype variation in behavioural traits (nest-holder versus floater; sneaker versus satellite), no significant differences have been found within the morphotype for both T and KT levels in two different studies (Oliveira *et al.*, 2001b; Ros *et al.*, 2006a). Also, Oliveira *et al.* (2001b) found in nest-holders some weakly negative correlations between androgen levels and several reproductive behaviours.

In a field study where nest-holders were treated with slow release implants filled with KT in oil or with oil only, sexual behaviour was not facilitated by the KT treatment (Ros *et al.*, 2004). However, KT males increased territorial aggression, responding to conspecifics at longer distances from the nest and chasing them until they were further from the nest than the control males (Ros *et al.*, 2004). In aquaria experiments, KT implanted satellites did not show any difference in the frequency of aggressive displays towards a mirror or of courtship displays towards females when compared with control animals (Oliveira *et al.*, 2001c). These results, in combination with the basal KT levels measured in satellites in the field, seems a paradox in the light of the positive effect of KT on territorial behaviour in nest-holders. Thus, variation in androgen levels seems to be more associated with morphological rather than behavioural differences between male morphotypes. Possibly, other neuroendocrine mechanisms may be more relevant in the mediation of behavioural variation between male tactics.

'A Fish Called Tootsie': Female-like Males in the Peacock Blenny

Male alternative reproductive tactics have been well characterised in the peacock blenny *Salaria pavo*. Ruchon *et al.* (1995)~~,~~ first described the occurrence of male alternative phenotypes in this species at a brackish lagoon in Maugio, France, and soon after, female-mimicry was identified as an alternative male mating tactic in a population at Ria Formosa, Portugal (Gonçalves *et al.*, 1996; Fig. 4.3.4g). The Ria Formosa population has since then been extensively studied and a number of interesting features have been described. Most data presented below on ART, thus, concerns this population but first~~,~~ a general review on the species reproductive biology and sexual behaviour is presented.

Sexual dimorphism is intense in *S. pavo*, with males being larger than females and having a well-developed head crest and an anal gland in the first two rays of the anal fin (Fishelson, 1963; Patzner *et al.*, 1986; Fig. 4.3.4h). The species reproductive biology follows the general pattern for blennies: the males defend a nest in a rock crevice or hole from which they attract the females; the females attach demersal eggs in a monolayer to the cavity walls; and parental care is provided exclusively by the male until egg hatching.

The patterns of sexual displays are very different between males and females. When approaching a nest, females court the males by beating the pectoral fins and opening and closing the mouth in synchrony while displaying a typical nuptial coloration that consists of contrasting dark and light bands across the anterior portion of the body (Fishelson, 1963; Patzner *et al.*, 1986). Males court the females usually from the nest by increasing the contrast between yellow and brown bands in the throat and head crest while performing intense lateral body jerkins. More rarely, the male leaves the nest and swims around the female while displaying body jerkins and then returns to the nest. In the nest, the male moves back and forth, alternating between protruding its head from the nest and going completely inside the nest. This behaviour is usually followed by the female further approaching the nest while courting and entering the nest (Fishelson, 1963; Patzner *et al.*, 1986; pers. obs.).

In the Ria Formosa, ecological constraints seem to promote both sex-role reversal and the occurrence of male alternative reproductive tactics. The species occurs in a mudflat area where the only hard substrates available for nests are artificial debris, such as bricks, used by clam culturists to delimit concessions (Fig. 4.3.4g). Most males nest in brick holes and do not defend any area around the nest. Several males are usually found reproducing in different holes of the same brick (range: 1-6 nesting males per brick, Almada *et al.*, 1994; Fig. 4.3.4i). Because nest availability is low, male-male competition for nests is high. Accordingly, at the peak of the breeding season~~,~~ there are many non-nesting floater males in reproductive conditions who are, on an average, smaller than the nesting males (Almada *et al.*, 1994; Oliveira *et al.*, 1999). The scarcity of nests is likely to bias the operational sex ratio towards females (see Almada *et al.*, 1995; Oliveira *et al.*, 1999). Accordingly, sex-roles are reversed and females take the initiative in courtship and exhibit courtship displays more often than males (Almada *et al.*, 1995; Fig. 4.3.4k).

Small males are unable to compete for the few available nests and adopt an alternative reproductive tactic. These males are morphologically similar to females and mimic the complex female reproductive displays and nuptial coloration in order to approach the nesting males and achieve parasitic fertilisations of eggs (Gonçalves *et al.*, 1996; Fig. 4.3.4k). Nesting males are deceived by these parasitic males, as is suggested by the fact that they attack and court matched for size parasitic males and females with the same probability (Gonçalves *et al.*, 2005). However, as body size increases, parasitic males are more attacked and less courted by nesting males when compared with matched for size females, suggesting larger parasitic males are poorer female-mimics (Gonçalves *et al.*, 2005). The cues used by nesting males for better discrimination at larger sizes are still unclear.

Evidence that both sex-role reversal and male alternative reproductive tactics are promoted by nest shortage comes from a comparative study between this population and a population from the Adriatic. The Adriatic population occurs in a rocky shore area where nest availability is high. Males reproduce in rock crevices and actively defend the area around the nest. Sex-roles are not reversed, as males take the initiative in courtship and perform more courtship displays than females and, in comparison with the Ria Formosa population, males leave the nest to court females more often (J. Saraiva and R.F. Oliveira, unpublished data). These differences suggest that nest availability modulates the frequency of male and female sexual displays. Because nest availability is high in the Adriatic, small males can acquire nests and try to attract females. In accordance, the average size of nesting males is smaller in the Adriatic and some nesting males are as small as parasitic males in the Ria Formosa (J. Saraiva, and R.F. Oliveira, unpublished data). Nevertheless, some small males lacking secondary sexual characters with enlarged testis occur in a small proportion, suggesting male ART are not completely absent in the Adriatic (J. Saraiva, and R.F. Oliveira, unpublished data). These data suggest that ART in *S. pavo* is condition-dependent.

In the Ria Formosa, parasitic males show a strong site fidelity, usually associating with only one brick that contains one or more nesting males throughout the breeding season. Bricks that on an average receive more eggs throughout the breeding season have more and larger parasitic males attached (Gonçalves *et al.*, 2003). This suggests that either the parasitic males compete between them for access to the most successful breeding sites or that females prefer to spawn in nests that have more and larger parasitic males attached.

The parasitic reproductive tactic is only adopted during the males' first breeding season, as has been revealed by an ongoing mark-recapture study. All parasitic males recaptured in their second breeding season had well-developed male secondary sexual characters and some successfully established nests and guarded eggs (T. Fagundes, J. Saraiva, D. Gonçalves and R.F. Oliveira, unpublished data). Alternatively~~,~~ to this ontogenetic reproductive route, some males apparently skip reproduction during the first year, having poorly developed testis during the breeding season (Gonçalves *et al.*, 1996). These males also develop male characters and may succeed in establishing a nest in the following breeding season (T. Fagundes, J. Saraiva, D. Gonçalves and R.F. Oliveira, unpublished data). The mechanisms influencing the young males' decision to reproduce or not during their first breeding season remains unknown.

Males adopting sequential alternative reproductive tactics undergo major morphologic, physiologic and behavioural changes. Morphologically, body size increases, the head crest and the anal gland develop and a gland also develops in the testis. This testicular gland has been shown to be the main source of gonadal androgens (Oliveira *et al.*, 2001a) and, accordingly, nesting males have higher circulating levels of both T and KT when compared to parasitic males (D. Gonçalves, M. Teles, J. Alpedrinha and R.F. Oliveira, unpublished data). The increase in circulating androgens may be responsible for some of the morphological changes taking place during tactic transition, as suggested above for *P. parvicornis*. The administration of KT implants to sneakers promoted the development of the genital papilla and anal gland within one week (Oliveira *et al.*, 2001d), while T was less effective in promoting the development of these male characters (Gonçalves *et al.*, 2007). In accordance, the KT/T ratio gradually increases from sneakers to transitional males to nest-holders (unpublished data).

Androgens have also been shown to mediate behavioural changes in *S. pavo*. Parasitic males switch from courting nesting males with female-like displays during their first breeding season into courting females with typical male behaviours during the remaining reproductive periods. Thus, both a defeminisation and a masculinisation of the males' reproductive behaviour occur during tactic switching. The administration of both KT and T to parasitic males decreases the frequency of female-like displays (Oliveira *et al.*, 2001d; Gonçalves *et al.*, 2007). However, the mechanism through which androgens exert this action remains unknown. One hypothesis is that androgens modulate AVT circuits, as shown across vertebrates (e.g., De Vries and Panzica, 2006). Both parasitic males and females express more AVT mRNA in a per-cell basis in the pre-optic area (Grober *et al.*, 2002) and AVT administration promotes female displays in these two morphs (Carneiro *et al.*, 2003). Thus, a down regulation of AVT as a consequence of an increase in circulating androgens during tactic switching could be a possible mechanism underlying the parasitic males' defeminisation.

The influence of androgens on behavioural defeminisation contrasts with the results found for behavioural masculinisation where androgen implants failed to promote male behaviors in parasitic males (Oliveira *et al.*, 2001d; Gonçalves *et al.*, 2007). There are three possible interpretations for these results: (1) the time length of the endocrine manipulations (8 days) was insufficient to behaviourally masculinise parasitic males; (2) other neuroendocrine mechanisms underlie behavioural masculinisation; (3) changes in other components of the steroid pathway (e.g., steroid receptors, steroidogenic enzymes) are necessary for androgens to exert a masculinising effect. Further experiments should allow testing these hypotheses.

TOWARDS A UNIFYING THEORY FOR THE INTRA-SEXUAL VARIATION IN MALE REPRODUCTIVE BEHAVIOUR IN FISH: LESSONS FROM BLENNIES

The comparative approach to the understanding of the proximate mechanisms of intra-sexual variation in fish is a valuable tool for various reasons. First, it promotes the development of a conceptual framework to explain these phenomena that is not species-centered. One major problem in this area is that a lot of research effort has been invested in only a reduced number of species, so that the information available for these few species abounds in detail but it tends to be extrapolated, as is valid to the whole group of teleosts. Therefore, the collection of data on different species exhibiting alternative tactics contributes to the awareness that similar functional phenomena may have different underlying mechanisms and promotes the search for commonalities among species. In turn, these prompt the generation of hypotheses that organise the observed variation and, thus, contribute to the development of a framework that explains the evolution of proximate mechanisms underlying alternative tactics. In the following paragraphs, we will try to develop such a framework based on the comparative results summarised in this chapter.

An important distinction that should be made—when studying the proximate mechanisms of intra-sexual variation in male reproduction—is whether the alternative phenotypes diverge only in terms of behavioural traits or if differences in the expression of morphological and anatomical traits are also present.

Empirical evidence suggests that major changes in reproductive behaviour may occur without significant variation in circulating hormone levels. On the contrary, morphological and anatomical modifications in reproductive structures are thought to be more dependent on hormonal effects (see below). Thus, behaviour is expected to be more labile than morphology and anatomy and the mechanisms underlying the expression of behavioural variation should be more flexible than those underlying morphological and anatomical variations. It further follows that alternative reproductive tactics that only involve differences in behaviour are expected to differ in the activation of different neural substrates but not necessarily to display different hormone profiles. On the other hand, alternative reproductive phenotypes that diverge in morphological traits (i.e., intra-sexual polymorphisms) are expected to also have different hormone profiles. It could be argued that differences in hormone levels should only be present at the period of the differentiation of the tactic, if their effects were to be organisational. However, there are several pieces of evidence suggesting that androgen-dependent traits, typical of bourgeois males, need the continuous exposure to androgens in order to be maintained. For example, in species that seasonally reproduce the development of male secondary sexual characters is maximum during the breeding season and coincident with an increase in circulating androgen levels (e.g., Mayer *et al.*, 1990). Also, it has long been demonstrated that castration induces the regression and exogenous administration of androgens restores the development of male secondary sexual characters (e.g., sonic muscles in vocalising males of *Porichthys notatus*, Brantley *et al.*, 1993b; but see also Modesto and Canário, 2003).

Below, the hypothesis that the differences between male morphs among different phenotypic traits (i.e., behavioural, morphological and gonadal) may be ruled by different proximate mechanisms will be evaluated.

In order to compare the magnitude of the differences between alternative male types in androgen levels and in the forebrain AVT system, we have calculated the effect sizes for each of the variables for the two species studied (Thalheimer and Cook, 2002; Fig. 4.3.5). We have used Cohen's d as a measure of effect size since it has become the standard measure in the literature, enabling the comparison of the data presented here with other published studies. Moreover, the fact that Cohen's d values give per se an indication of the magnitude of the effect size (i.e., 0.2 = small; 0.5 = medium; 0.8 = large) allows us to interpret the values obtained by comparing them to these known benchmarks (Rosnow and Rosenthal, 1996; Rosnow *et al.*, 2000; Thalheimer and Cook, 2002). To facilitate the reading of the following sections, we have also compiled an overview of the behavioural, morphological and gonadal differences between the alternative morphs for the species reported here (Table 4.3.2).

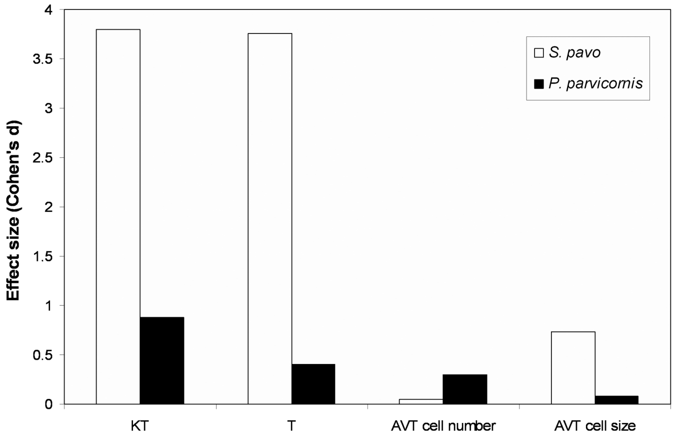

Fig. 4.3.5 The magnitude of the differences between alternative male types of *P. parvicornis* and *S. pavo* in androgens (KT and T) and in forebrain AVT (cell number and cell size) was calculated by computing the Cohen's d effect size measure.

Table 4.3.2 Behavioural, morphological and gonadal characteristics of mature males in *Parablennius parvicornis* and *Salaria pavo*

	Parablennius parvicornis				*Salaria pavo*	
	M–		M+			
	Sneakers	*Satellites*	*Floaters*	*Nesting males*	*Sneakers*	*Nesting males*
Behavioural	Roam around several pools with a cryptic behaviour in order to enter nests and fertilise the eggs.	Help in territory defense and female attraction to attain a privileged position for parasitic fertilisations.	Non-territorial, are looking for a vacant territory to establish a nest.	Actively defend the territory and court females. Provide parental care to the eggs.	Assume a female-like behaviour in order to approach the nests of nesting males and parasitically fertilise the eggs.	Adopt a passive role during courtship, rarely court the females. Do not defend a territory around the nest. Provide parental care to the eggs.
Morphological	The smallest of all male classes. Do not express male secondary sexual characters.	Do not express male secondary sexual characters.	Do not display the nesting male nuptial coloration. Smaller than nesting males. Intermediate pattern of development of male secondary sexual characters.	Have a distinct dark-brown coloration, a distinct head hump and an anal gland in the first two rays of the anal fin.	Much smaller than nesting males. Do not display male secondary sexual characters.	Have a well-developed head-crest and an anal gland in the first two rays of the anal fin.
Gonadal	Enlarged testis in relation to M+ males. The testicular gland is either absent or vestigial.	Enlarged testis in relation to M+ males. The testicular gland is absent or vestigial.	Smaller testis than M– males. The testicular gland is present.	Smaller testis than M– males. The testicular gland is present.	Enlarged testis in relation to nesting males. The testicular gland is absent or vestigial.	Smaller testes than sneakers. The testicular gland is present.

Hormones and Differences between Alternative Phenotypes in Secondary Sex Characters

Ketotestosterone (KT) has been demonstrated to be the most potent androgen in the induction of secondary sex characters in male teleosts (Borg, 1994). Thus, differences in KT levels may be less important in species with alternative tactics that lack major tactic-specific morphological specialisations (e.g., the expression of male secondary sex characters in bourgeois males). Between the two species studied here *S. pavo,* that displays a higher degree of phenotypic specialisation of the alternative tactics, also exhibits a larger effect size in androgen levels between alternative male types (Fig. 4.3.5). These results suggest that differential KT levels are necessary in order to induce or to maintain tactic-specific morphological characters but may not be needed when the alternative tactics only differ in behavioural traits. If this hypothesis is correct an association between the degree of dimorphism between the alternative male phenotypes and the differential variation in KT levels between alternative morphs is to be expected. In our two study species, major differences in KT levels occur between morphotypes that differ in the development of secondary sexual characters (i.e., M+ versus M– males in *P. parvicornis*, and sneakers versus nesting males in *S. pavo*) while in morphotypes that only differ in behaviour (i.e., sneakers versus satellites and floaters versus nesting males in *P. parvicornis*) KT levels are similar (Oliveira *et al.*, 2001a, b and unpublished data). Additionally, from all species for which androgen levels are known when the ART involves a morphological intra-sexual dimorphism (apart from differences in body size), the KT (but not T) levels are higher in the bourgeois than in the parasitic male, and irrespective of the ART they display (Oliveira, 2006). This suggests a parallel to the androgen correlates of sex-change in fish in that androgens are thought to play a major role in the morphological differentiation during sex-change but are not essential for the occurrence of behavioural sex-change (Godwin *et al.*, 1996; Grober, 1998; Reavis and Grober, 1999).

Hormones and Differences between Alternative Phenotypes in Reproductive Behaviour

Several studies suggest that reproductive behaviour may change dramatically without major variations in circulating hormone levels. For example, in the protogynous blue-head wrasse, *Thalassoma bifasciatum*, the experimental removal of a territorial male allows large females to attain social dominance and to change sex. In this experimental setting, ovariectomised females do not differ from sham-operated females in the sense that they also change their behaviour and display the full suite of male reproductive behaviours (Godwin *et al.*, 1996). This suggests that the regulation of reproductive behaviours is more dependent on other neuroendocrine mechanisms and less on gonadal hormones. Brain neuropeptides systems are likely candidates for this regulation. In the above example of *T. bifasciatum*, female-to-male behavioural switch is closely followed by major changes in hypothalamic AVT mRNA levels (Godwin *et al.*, 2000) and blocking AVT action (with an AVP V1 receptor antagonist) inhibits females from behaviourally changing into males (Semsar and Godwin, 2004). The AVT system has also been studied in our two blenniid species in relation to reproductive behaviour. The asymmetry in the expression of courtship behaviour between alternative morphs is larger in *S. pavo* than in *P. parvicornis* (Table 4.3.2). In the Ria Formosa population of *S. pavo*, nest-holders lack male courtship behaviour (or display it in a low frequency), while sneaker males display female-like courtship behaviours. In *P. parvicornis,* there is only a quantitative differentiation in reproductive behaviour between satellites and nesting males, as satellites also participate in territory defense and female attraction (Santos, 1985b; Oliveira *et al.*, 2002). Interestingly, the effect size is largest for AVT cell size in *S. pavo* (Fig. 4.3.5) while in *P. parvicornis* AVT cell size presents a negligible effect size and only a small effect size is detected for AVT cell number (Fig. 4.3.5). These results, together with the fact that in some species AVT promotes female displays (e.g., *S. pavo*) and in others male displays (e.g., *T. bifasciatum*), suggest that AVT is closely associated with the general expression of courtship behaviour rather than specifically with male or female sexual displays.

Nevertheless, sex steroids have also been shown to significantly influence reproductive displays in species with alternative reproductive tactics. For example, androgens (both T and KT) inhibit the expression of female-like displays in sneakers of *S. pavo* (Oliveira *et al.*, 2001d; Gonçalves *et al.*, 2007). As AVT promotes these displays (Carneiro *et al.*, 2003), it seems possible that androgens act on these behaviours through the suppression of the AVT system. Similar results have been reported for the midshipman *Porichthys notatus* (Goodson and Bass, 2000). Thus, sex steroids do not seem to be critical to induce or maintain differences in reproductive behaviour between males displaying alternative tactics but are likely to act as modulators of the neural substrates underlying the expression of these behaviours.

Hormones and Differences between Alternative Phenotypes in Gonadal Allocation

Apart from gonadotrophins, androgens also participate in the regulation of spermatogenesis, with KT stimulating later stages of the process and T being involved in the negative feedback mechanisms needed to control KT-dependent spermatogenesis. Thus, a balance between T and KT is needed for the control of spermatogenesis (Schulz and Miura, 2002). Therefore, the ratio between KT and T should be more informative than absolute KT or T levels. If the KT:T ratio is computed for the species for which both androgen levels and GSI values are available (n = 9 species) a trend for the magnitude of the difference in the KT:T to be negatively correlated with the magnitude of the difference in GSI values between alternative male types is found (Oliveira, 2006). In other words, in species for which the magnitude of the difference in KT levels between bourgeois and parasitic is larger, there is a smaller difference in relative gonad size. This also means that among parasitic males, a higher GSI is associated with a lower KT:T ratio, which probably allows them to have larger testes without a linked expression of the secondary sex characters and of bourgeois male behaviour.

To summarise, the relative importance of different physiological mechanisms for the differentiation of tactic-specific traits might vary among behavioural, morphological and gonadal traits and, therefore, the classic paradigm of androgens controlling the expression of the whole set of reproductive characters that characterise each tactic should be questioned (Fig. 4.3.3).

Finally, it should also be mentioned that the decoupling of different male traits in parasitic males may be achieved by other mechanisms apart from differences in hormone levels, namely by varying the local micro-environments in the different target tissues, due to differential expression of receptors or to differential levels of activity of catabolic enzymes that modulate the availability of the active hormone to specific targets (e.g., 11-β-hydroxilase and 11-β-HSD, that metabolise T into KT, are key steps in the expression of male secondary sex characters, spermatogenesis and the modulation of the expression of reproductive behaviour in male teleosts, Borg, 1994). This focus on target tissues has rarely been used when studying the mechanisms of intra-sexual variation in reproduction. One of the rare examples of such an approach is a study on the relative levels of brain steroid receptors between alternative reproductive phenotypes in the protogynous wrasse, *Halichoeres trimaculatus*, where it was found—using competitive RT-PCR—that the levels of AR transcripts were significantly higher in the brain of terminal phase males than in initial phase males (Kim *et al.*, 2002). No other significant differences in gene expression were observed either for AR in the gonads or for ER both in the brain and in the gonads. Thus, by varying the expression of AR in specific tissues (brain versus gonad), bourgeois males (in this case, terminal phase males) can both increase their sensitivity to circulating androgen levels in specific targets (the brain) and, simultaneously, allow a compartmentalisation of the effects of androgens, so that the potential deleterious effects of androgens can be avoided by varying AR densities in different tissues (Ketterson and Nolan Jr., 1999). This mechanism hypothetically makes it possible to activate the expression of an androgen-dependent reproductive behaviour in bourgeois males without having the associated costs of increasing spermatogenesis or the expression of a sex character, since the androgen action can be independently modulated at each compartment (brain versus gonad versus morphological secondary sex character). Studies focusing on target tissues are, thus, a major avenue for future research in this area.

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