

# Snails and their trails: the multiple functions of trail-following in gastropods

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## ABSTRACT

Snails are highly unusual among multicellular animals in that they move on a layer of costly mucus, leaving behind a trail that can be followed and utilized for various purposes by themselves or by other animals. Here we review more than 40 years of experimental and theoretical research to try to understand the ecological and evolutionary rationales for trail-following in gastropods. Data from over 30 genera are currently available, representing a broad taxonomic range living in both aquatic and terrestrial environments. The emerging picture is that the production of mucus trails, which initially was an adaptation to facilitate locomotion and/or habitat extension, has evolved to facilitate a multitude of additional functions. Trail-following supports homing behaviours, and provides simple mechanisms for self-organisation in groups of snails, promoting aggregation and thus relieving desiccation and predation pressures. In gastropods that copulate, trail-following is an important component in mate-searching, either as an alternative, or in addition to the release of water- or air-borne pheromones. In some species, this includes a capacity of males not only to identify trails of conspecifics but also to discriminate between trails laid by females and males. Notably, trail discrimination seems important as a pre-zygotic barrier to mating in some snail species. As production of a mucus trail is the most costly component of snail locomotion, it is also tempting to speculate that evolution has given rise to various ways to compensate for energy losses. Some snails, for example, increase energy intake by eating particles attached to the mucus of trails that they follow, whereas others save energy through reducing the production of their own mucus by moving over previously laid mucus trails. Trail-following to locate a prey item or a mate is also a way to save energy. While the rationale for trail-following in many cases appears clear, the basic mechanisms of trail discrimination, including the mechanisms by which many snails determine the polarity of the trail, are yet to be experimentally determined. Given the multiple functions of trail-following we propose that future studies should adopt an integrated approach, taking into account the possibility of the simultaneous occurrence of many selectively advantageous roles of trail-following behaviour in gastropods. We also believe that future opportunities to link phenotypic and genotypic traits will make possible a new generation of research projects in which gastropod trail-following, its multitude of functions and evolutionary trade-offs can be further elucidated.

*Key words:* communication, gastropod, homing, mate search, mucus, pheromone, self-organisation.

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## I. INTRODUCTION

Trail-following, where individuals follow the tracks or paths of other individuals, occurs in many animal phyla and has almost certainly evolved many times. The known or perceived roles of this behaviour differ among taxonomic groups (Table 1) and this may be related to specific selection pressures for trail-following behaviour, and the mechanisms by which trail-following occurs. Many marine mammals, for example, locate their prey through hydrodynamic trails that are prey-generated (Dehnhardt *et al.*, 2001; Gläser *et al.*, 2011). This, however, appears to be a functionally different form of trail-following to that employed by ants optimising food resources through processes of self-organisation *via* pheromone trails (reviewed by Bonabeau *et al.*, 1997), or gastropod snails following mucus trails that contain mate-specific cues (Erlandsson & Kostylev, 1995; Ng *et al.*, 2011). While much work exists on the function of trail-following in social insects [including isolation and identification of pheromones (e.g. Bordereau *et al.*, 2010), self-organisation patterns (e.g. Bonabeau *et al.*, 1997) and roles of worker ants in maintaining trails (e.g. Evison, Hart & Jackson, 2008)], few studies have focused on other taxa, other than to acknowledge that trail-following occurs and to suggest adaptive benefits for this behaviour (Table 1).

One taxon where trail-following is widely acknowledged to occur is the Gastropoda (see Section II). The role of trail-following in gastropods appears multifaceted, and has attracted attention perhaps because of the obvious nature of the mucus trails and the high cost of mucus production. During locomotion, gastropods exert stresses on a thin (10–20  $\mu\text{m}$ ) layer of secreted pedal mucus, which acts as a glue and a lubricant (Denny & Gosline, 1980; Denny, 1980b). As the animal moves, the mucus left behind forms

a trail that shows the ‘history’ of the individual’s spatial movement patterns.

The morphology of the trail has received little attention, but in the intertidal prosobranch *Littorina littorea*, the trail has a convex profile, approximately 35  $\mu\text{m}$  at the centre and about 20  $\mu\text{m}$  at the edges (Davies & Blackwell, 2007). Gastropod pedal mucus largely consists of water (typically > 80%) and mucin or mucin-like carbohydrate-protein complexes (proteoglycans or glycosaminoglycans), which produce its characteristic sticky properties (see reviews by Davies & Hawkins (1998) and Smith (2002, 2006), for details on the composition and properties of gastropod mucus). Since mucus has a significant organic component, leaving a mucus trail places a considerable energetic burden on gastropods (Hawkins & Hartnoll, 1983; Davies & Hawkins, 1998; Table 2). Pedal mucus has calorific values of 9–24  $\text{kJ g}^{-1}$  dry mass (Calow, 1974; Davies, Hawkins & Jones, 1990a). To put this into context, molluscan (limpet) somatic tissue has a calorific value of approximately 20  $\text{kJ g}^{-1}$  (Wright, 1977), similar to that of secreted pedal mucus. In the intertidal gastropod *Littorina littorea*, for example, Davies, Jones & Hawkins (1992b) demonstrated that the cost of mucus production is much greater (35 $\times$ ) than the metabolic cost of locomotion and Denny (1980a) calculated that using mucus as a means of locomotion is an order of magnitude more expensive than any other mode.

An obvious question, therefore, is why do gastropods use such an energetically expensive form of locomotion? Many creeping organisms, for example annelids, can propel themselves without the need of mucus. It has been suggested that gastropods evolved from a platyhelminth-like ancestor that was mucus-coated and moved using ciliary locomotion within a secreted mucus film (Wilmer, 1990). Wilmer (1990) suggested that such locomotion would only be efficient for

Table 1. Examples of the occurrence and proposed adaptive benefits (i.e. experimentally supported functions) of trail-following in a range of non-gastropod taxa (for gastropod taxa see Table 3)

Animal phylum	Taxon/species	Proposed benefit (s)	References
Proteobacteria	Myxobacteria	Enhanced locomotion and aggregation	Burchard (1982) and Stevens (1995)
Platyhelminthes	<i>Platydemus manokwari</i>	Location of prey	Iwai <i>et al.</i> (2010)
Nemertina	<i>Paranemertes peregrina</i>	Location of prey	Amerongen & Chia (1982)
Mollusca	Chitons	Homing	Chelazzi <i>et al.</i> (1989, 1990)
Annelida	<i>Lumbricus terrestris</i>	Location of mate	Nuutinen & Butt (1997)
Arthropoda	Leeches	Location of prey	Kutschera <i>et al.</i> (2007) and Lai <i>et al.</i> (2011)
	Copepods	Location of mate	Weissburg <i>et al.</i> (1998) and Yen <i>et al.</i> (2011)
	Social insects (ants, termites and bees)	Location of food and nest	Bonabeau <i>et al.</i> (1997) and Jarau <i>et al.</i> (2010)
	Moths	Location of mate	Farkas & Shorey (1972) and Farkas <i>et al.</i> (1974)
	Beetles	Location of host	Cammaerts <i>et al.</i> (1990) and Quinet & Pasteels (1995)
Chordata	Caterpillars of non-social insects	Location of food and aggregation	Capinera (1980), Fitzgerald (1993), Ruf <i>et al.</i> (2001) and Pescador-Rubio <i>et al.</i> (2011)
	Lizards	Location of prey	Garrett <i>et al.</i> (1996)
	Snakes	Location of prey and mate	Gehlbach <i>et al.</i> (1971), Furry <i>et al.</i> (1991) and Webb & Shine (1992)
	Marine mammals	Location of prey	Dehnhardt <i>et al.</i> (2001) and Gläser <i>et al.</i> (2011)
	Rodents	Spatial navigation	Jamon (1994) and Lavenex & Schenk (1998)

Classification of animal phylum is based on Cavalier-Smith (1998). Where there is a single example within a taxon, the species name is given; the common name is given where there are multiple examples.

Table 2. The measured energetic burden of pedal mucus in gastropods

Species	Habitat	Energetic burden (%)	Reference (s)
<i>Hydrobia ventrosa</i>	Brackish water (estuary and lagoon)	9 <sup>a</sup>	Kofoed (1975)
<i>Cepaea nemoralis</i>	Terrestrial	~12 <sup>b</sup>	Richardson (1975)
<i>Ilyanassa obsoleta</i>	Marine (intertidal and subtidal)	23 <sup>c</sup>	Edwards & Welsh (1982)
<i>Haliotis tuberculata</i>	Marine (intertidal and subtidal)	23–29 <sup>c</sup> (dependent on size)	Peck <i>et al.</i> (1987)
<i>Patella vulgata</i>	Marine (intertidal)	23–31 <sup>c</sup> (dependent on population)	Davies <i>et al.</i> (1990a) and Davies & Hawkins (1998)
<i>Buccinum undatum</i>	Marine (intertidal and subtidal)	11 <sup>c</sup>	Kideys & Hartnoll (1991)
<i>Concholepas concholepas</i>	Marine (subtidal)	6–20 <sup>d</sup> (dependent on size)	Navarro & Torrijos (1995)
<i>Lottia kagamogai</i>	Marine (intertidal)	48 <sup>c</sup>	Niu <i>et al.</i> (1998)

Calculated as a proportion of either:

<sup>a</sup>Assimilated carbon.

<sup>b</sup>Assimilated energy.

<sup>c</sup>Consumed energy.

<sup>d</sup>Absorbed energy.

small animals; increased size only being possible when alternative forms of locomotion within the mucus film evolved, as seen in modern-day gastropods. One benefit of using mucus is that its adhesive properties allow snails to attach themselves firmly (adhesion is also achieved by suction, e.g. Smith, 2002) and hence can locomote on vertical surfaces and upside-down. These simple benefits allow animals to extend their habitat use into complex three-dimensional and dynamic (e.g. wave-swept) environments, and may have driven the evolution of locomotion on mucus, and hence the laying of mucus trails. Once laid, however, these trails can have other benefits to the trail-layer (see Section II) and it may be that post-deposition functions not associated with locomotion justify the high cost of mucus production.

Herein we review the current state of research on gastropod trail-following; from its functional significance to

the possible mechanisms driving this behaviour, and present a synthesis of the ecological and potential evolutionary significance of trail-following behaviour. We also highlight the limited studies on trail-following mechanisms in gastropods to encourage further research into this particular area. It is hoped that this synthesis will stimulate new research into trail-following in gastropods and other taxa.

## II. THE MULTIPLE ROLES OF TRAIL-FOLLOWING IN GASTROPODS

### (1) Homing

Some gastropods and chitons (Class Polyplacophora) return to specific resting positions after feeding excursions,

a behavioural pattern termed 'homing' (see reviews by Underwood, 1979; Branch, 1981; Hawkins & Hartnoll, 1983; Chelazzi, Focardi & Deneubourg, 1989; Cook, 2001). Solitary homers are mostly limpets living on rocky shores that have individual-specific resting sites of varying temporal persistence, termed 'home scars', onto which their shell typically fits snugly (Ohgushi, 1954; Cook, 1969; Branch, 1975; Connor, 1986). By contrast, collective homers share refuges, in which many individuals aggregate, usually around holes or in crevices (Cook, 1979; McFarlane, 1980; Chelazzi, Innocenti & Della Santina, 1983; Skov *et al.*, 2010).

Trail-following has frequently been reported in both solitary and collective homers, but it is often described as a complementary or subordinate mechanism rather than a key mechanism in driving homing (Chase & Croll, 1981; Chelazzi, Della Santina & Vannini, 1985; Chelazzi, Le Voci & Parpagnoli, 1988; Cook, 1992). Many homing species, for example, do not necessarily return to their resting sites *via* the same route as they left; and some homing species, when artificially displaced, are still able to find their way back (Stephenson, 1936; Beckett, 1968; Cook *et al.*, 1969; Thomas, 1973), suggesting that trail-following is not the primary means by which these animals locate their homes. Some of these studies, however, overlooked the fact that trails may persist over long periods (e.g. Davies, Hawkins & Jones, 1992a) and that homing individuals may use old, previously laid, trails to find their way home (Cook, 1969, 1971; Chelazzi *et al.*, 1985). Interruption of trails by various methods such as chiseling or washing the rock have had varying degrees of influence on homing success (Galbraith, 1965; Jessee, 1968; Cook *et al.*, 1969; McFarlane, 1980), which suggests the presence of species-specific variation in dependence on the use of mucus trails in homing behaviour. Homing is relatively common in intertidal species, perhaps because the selective pressures on homing to a fixed location, as a mechanism to prevent increased desiccation or predation risk, are greater in this environment. Consequently, and because of its accessibility for observation and manipulative experiments, the intertidal zone has yielded most of the evidence for the functional significance of trail-following in gastropod homing. Funke (1968), for example, showed that replacing the mucus trail of an individual limpet, *Patella vulgata*, with that of a conspecific could disrupt homing behaviour, suggesting that limpets recognize individual trails, a trait also identified in the collective homer, *Onchidium verruculatum* (McFarlane, 1980), and in the non-homing *Littorina littorea* (Davies & Beckwith, 1999). The incorporation of individual-specific cues into mucus trails suggests that some homers rely, to a certain degree, on contact chemoreception to return to their individual homes. Further evidence for this is provided by studies showing that many homing species can determine polarity (i.e. directionality) of the trails they follow to return home (see Section III.3).

The significance of trail-following for homing behaviours cannot, however, be properly interpreted without an understanding of the effects of other factors that have been shown to influence trail-following [e.g. humidity and

type of microhabitat (McFarlane, 1980) and wind direction (Cook, 1980, 1992; Chase & Croll, 1981)]. The slug, *Limax pseudoflavus*, for example, detects air-borne chemical cues carried by the prevailing wind from its resting sites (i.e. *via* distance chemoreception) to aid homing, but when changes in wind direction disrupt these cues, trail-following (i.e. contact chemoreception) may become the dominant mechanism (Cook, 1980, 1992). While it appears that mucus-trail-following is only one of a variety of tools that gastropods use to return home, it is clear that in some species and under some conditions, trail-following has an important role in homing behaviour.

## (2) Mate location and communication

### (a) Locating the right species

Mate location in many aquatic gastropods is mediated by water-borne chemicals (Cate, 1968; Painter *et al.*, 1998; Moomjian, Nystrom & Rittschof, 2003; Takeichi, Hirai & Yusa, 2007) and in terrestrial gastropods by air-borne chemicals (Chase *et al.*, 1978; Cook, 1992). Mucus-trail-following may be an alternative or a complementary mate-searching strategy to these chemical cues (Table 3). In the mostly hermaphroditic opisthobranchs and pulmonates (Heller, 1993), mate-searching involves discrimination of species. Conspecific trail-following is commonly observed in these gastropods and, as it often results in courtship, this behaviour has been associated with mate-searching (Quick, 1960; Lowe & Turner, 1976; Cook, 1977, 1992; Todd, 1977, 1979; Hirano & Inaba, 1980; Hadfield & Switzer-Dunlap, 1984; Leonard & Lukowiak, 1985; Wareing, 1986; Nakashima, 1995; Reise, 2007). Few studies have, however, provided experimental data to confirm this link, although Townsend (1974) demonstrated that sexually aroused freshwater pulmonates, *Biomphalaria glabrata*, showed a higher degree of conspecific trail-following than non-aroused conspecifics. Nakashima (1995) also provided experimental evidence for trail-following as a means to locate mates in opisthobranchs, showing that in the mating season two nudibranch species (*Dendrodoris nigromaculata* and *D. nigra*) relied on mucus trails rather than on water-borne chemicals to locate conspecifics, and were able to discriminate between conspecific and heterospecific trails. There is also good evidence for species-specific trail cues in dioecious prosobranch gastropods. Ng *et al.* (2011), for example, demonstrated that males of two mangrove littorinids, *Littoraria ardouiniana* and *L. melanostoma*, were able to discriminate conspecific from heterospecific females by their mucus trails. Individuals of *Nassarius vibex* can also distinguish conspecific from heterospecific trails (Trott & Dimock, 1978). The incorporation of species-specific cues into mucus trails is, therefore, advantageous in facilitation of mate-searching in both hermaphroditic and dioecious gastropods.

### (b) Locating the right sex

For many prosobranch gastropods that have separate sexes (Heller, 1993), mate location will be optimized if males not

Table 3. A summary of the gastropod genera known to exhibit mucus trail-following behaviour and its proposed benefits

Genus	Family	Sexual mode	Habitat	Proposed benefit (s)	References
<b>Prosobranchs</b>					
<i>Collisella</i>	Lottidae	Gonochoric	Marine (intertidal)	Homing	Hewatt (1940)
<i>Echinolittorina</i>	Littorinidae	Gonochoric	Marine (intertidal)	Nutritional benefit <sup>a</sup>	Connor & Quinn (1984) and Connor (1986)
<i>Ilyanassa</i>	Nassariidae	Gonochoric	Marine (intertidal and subtidal)	Aggregation <sup>b</sup> Unknown <sup>c</sup>	Stafford <i>et al.</i> (2007, 2011) Trott (1978), Trott & Dimock (1978), Dunn (1982) and Bretz & Dimock (1983)
<i>Littoraria</i>	Littorinidae	Gonochoric	Marine (intertidal)	Location of mate <sup>a</sup> Aggregation	Ng <i>et al.</i> (2011) Alfaro (2007)
<i>Littorina</i>	Littorinidae	Gonochoric	Marine (intertidal)	Location of mate <sup>d,e</sup>	Peters (1964), Raftery (1983), Erlandsson & Kostylev (1995), Erlandsson (2002), Johannesson <i>et al.</i> (2008, 2010) and Saltin (2010)
<i>Lotia</i>	Lottidae	Gonochoric	Marine (intertidal)	Nutritional benefit <sup>a</sup>	Davies & Beckwith (1999) and Edwards & Davies (2002)
<i>Melarhaphe</i>	Littorinidae	Gonochoric	Marine (intertidal)	Energy saving <sup>a</sup> Unknown <sup>c</sup>	Davies & Blackwell (2007) Stirling & Hamilton (1986)
<i>Monodonta</i>	Littorinidae	Gonochoric	Marine (intertidal)	Homing	Wright (1977)
<i>Natica</i>	Naticidae	Gonochoric	Marine (intertidal)	Nutritional benefit <sup>a</sup>	Connor & Quinn (1984) and Connor (1986)
<i>Nassarius</i>	Nassariidae	Gonochoric	Marine (intertidal and subtidal)	Aggregation <sup>b</sup>	Stafford & Davies (2005)
<i> Nerita</i>	Neritidae	Gonochoric	Marine (intertidal)	Energy saving and/or nutritional benefit <sup>d</sup>	Hutchinson <i>et al.</i> (2007)
<i>Nodilittorina</i>	Littorinidae	Gonochoric	Marine (intertidal)	Location of prey	Gonor (1965)
<i>Olivella</i>	Olivellidae	Gonochoric	Marine (intertidal and subtidal)	Unknown <sup>c</sup>	Trott (1978) and Trott & Dimock (1978)
<i>Patella</i>	Patellidae	Gonochoric	Marine (intertidal)	Homing <sup>e</sup>	Chelazzi <i>et al.</i> (1983, 1985)
<i>Pomacea</i>	Ampullariidae	Gonochoric	Fresh water	Aggregation <sup>f</sup>	Chapman (1998)
<b>Opisthobranchs</b>					
<i>Barsatella</i>	Aplysiidae	Hermaphroditic	Marine (intertidal and subtidal)	Location of mate	Edwards (1968)
<i>Dendrodroris</i>	Dendrodrorididae	Hermaphroditic	Marine (intertidal and subtidal)	Homing <sup>e</sup>	Funke (1968) and Cook <i>et al.</i> (1969)
<i>Navanax</i>	Aglajidae	Hermaphroditic	Marine (intertidal and subtidal)	Unknown <sup>c</sup>	Taketchi <i>et al.</i> (2007)
<i>Onchidoris</i>	Onchidorididae	Hermaphroditic	Marine (intertidal and subtidal)	Aggregation and/or location of mate <sup>a</sup>	Lowe & Turner (1976)
<i>Sylocheilus</i>	Aplysiidae	Hermaphroditic	Marine (intertidal and subtidal)	Location of mate <sup>c</sup>	Nakashima (1995)
<b>Pulmonates</b>					
<i>Achatina</i>	Achatinidae	Hermaphroditic	Terrestrial	Location of prey	Paine (1963) and Blair & Seapy (1972)
<i>Biomphalaria</i>	Planorbidae	Hermaphroditic	Fresh water	Location of mate	Leonard & Lukowiak (1985)
<i>Deroceras</i>	Agriolimacidae	Hermaphroditic	Terrestrial	Location of mate	Todd (1979)
<i>Englandina</i>	Spiraxidae	Hermaphroditic	Terrestrial	Location of mate	Switzer-Dunlap & Hadfield (1979)
				Location of mate <sup>d</sup>	Chase <i>et al.</i> (1978)
				Location of mate <sup>d</sup>	Townsend (1974) and Bousfield <i>et al.</i> (1981)
				Location of mate	Reise (2007)
				Location of prey <sup>f,c</sup>	Clifford <i>et al.</i> (2003), Shabben <i>et al.</i> (2005), Davis-Berg (2012) and Holland <i>et al.</i> (2012)
				Location of mate <sup>a</sup>	Cook (1985a)

Table 3. (Cont.)

Genus	Family	Sexual mode	Habitat	Proposed benefit (s)	References
<i>Haplotrema</i>	Haplotrematidae	Hermaphroditic	Terrestrial	Location of prey <sup>d</sup>	Pearce & Gaertner (1996)
<i>Helix</i>	Helicidae	Hermaphroditic	Terrestrial	Unknown	Bailey (1989)
<i>Limax</i>	Limacidae	Hermaphroditic	Terrestrial	Homings <sup>e,a</sup>	Chelazzi <i>et al.</i> (1988) and Cook (1992)
				Location of mate <sup>a</sup>	Cook (1977, 1980)
<i>Mariella</i>	Ariophantidae	Hermaphroditic	Terrestrial	Unknown <sup>a</sup>	Ushadevi & Krishnamoorthy (1980)
<i>Mesodon</i>	Onchidiidae	Hermaphroditic	Terrestrial	Unknown <sup>f</sup>	Davis (2007)
<i>Onchidium</i>	Onchidiidae	Hermaphroditic	Marine (intertidal and subtidal)	Homings <sup>e</sup>	McFarlane (1980, 1981)
<i>Plysa</i>	Physidae	Hermaphroditic	Fresh water	Unknown <sup>c</sup>	Wells & Buckley (1972)
<i>Siphonaria</i>	Siphonariidae	Hermaphroditic	Marine (intertidal)	Homings <sup>e</sup>	Cook (1969, 1971) and Cook & Cook (1975)
				Location of mate	Hirano & Inaba (1980)

Superscripts indicate the method used to study trail-following (see Appendix for a detailed description of each method).

<sup>a</sup>Other methods.

<sup>b</sup>Computer models.

<sup>c</sup>T- or Y-maze.

<sup>d</sup>Coincidence index.

<sup>e</sup>Videography and spatial mapping.

<sup>f</sup>Perpendicular placement method.

The absence of a superscript indicates that the proposed benefit was not supported experimentally but was suggested based on field or laboratory observations.

only recognize the correct species but also the correct sex. High-shore littorinid snails have frequently been used as models to study the role of trail-following in mate location because these abundant snails are highly accessible and, in general, can be easily sexed by the presence or absence of a penis. In these snails males actively search for females and are generally able to discriminate conspecific females from conspecific males *via* their mucus trails (Erlandsson & Kostylev, 1995; Johannesson *et al.*, 2010; Ng *et al.*, 2011; but see Section II.2*d* for an intriguing exception in *Littorina saxatilis*). Owing to infrequent submersion by sea water, trail-borne cues may be more effective than water-borne cues in locating mates in these high shore species. Nonetheless, males of the freshwater snail species *Pomacea canaliculata* are attracted by water-borne cues from females, and both sexes follow mucus trails of the opposite sex, but females also follow trails laid by conspecific females, making it difficult to conclude whether trail-following is linked to mate-searching (Takeichi *et al.*, 2007). Despite such variation in behaviour it has frequently been suggested that sex-specific cues are incorporated in gastropod mucus trails (Table 3) and may play a crucial role in the reproductive success of at least some species.

### (c) Sexual selection

There is limited information on sexual selection through trail-following in gastropods but, again, the high-shore littorinid snails have proved a model study group. Trail-following to locate a mate has generally been regarded as a pre-courtship or pre-copulatory process, and only a few studies have examined the possibility of female quality assessment during trail-following. Males may, at the trail-following stage, be able to gain information about female quality such as body size or parasite infection, and thereby an indication of female fecundity, because fecundity increases with size and snails can become sterile in response to trematode infections (Hughes & Answer, 1982; Baur, 1992; Norton & Bronson, 2006). Gaining this type of information from female mucus trails will enable males to optimize costly mate-searching by choosing to follow the most fecund females.

In littorinids, males show a preference to mate more frequently, and copulate for longer, with larger females (Saur, 1990; Erlandsson & Johannesson, 1994; Zahradnik, Lemay & Boulding, 2008; Saltin, Schade & Johannesson, in press). Saltin (2010) reported that males of *Littorina fabalis* preferred to follow trails laid by females of a larger species, *L. obtusata*, over trails laid by smaller conspecific females. These results are puzzling, since the two species are genetically distinct and hybridization is not known (Kemppainen *et al.*, 2009). This example also illustrates the possibility that other beneficial functions of trail-following may be involved in this behaviour, making the identification of a single dominant function difficult. Nevertheless, this study showed that gastropod males are able to discriminate between trails from females of different sizes, a phenomenon also found in *Littoraria arduiniana* (T. P. T. Ng, unpublished observations) and probably in other gastropod taxa.

In addition to size, the mucus trails of females may carry signals of other aspects of mate quality. Male *Littorina littorea*, for example, are reluctant to follow mucus trails laid by females made sterile by trematode parasites (Erlandsson & Kostylev, 1995). This may be mediated through the male being able to detect the presence of parasites present in the mucus, as trematode cercariae have been identified in mucus trails laid by parasitized snails (Curtis, 1993; Davies & Knowles, 2001) demonstrating that male *L. littorea* avoid such 'contaminated' trails. Edwards & Davies (2002) showed that *L. littorea* can also detect the starvation level of conspecifics from their mucus trails, which also has implications for mate choice. Thus data from studies on littorinids suggest that sexual selection through mucus trail-following is feasible in gastropods.

#### (d) Sexual conflict

As discussed above, males of many species, including littorinids, can discriminate between female and male trails. In *Littorina saxatilis*, however, males do not differentiate between male and female trails (Johannesson *et al.*, 2010). This may be a result of sexual conflict over mating frequency. *L. saxatilis* lives at high densities and extreme polyandry has been recorded on the shore (Panova *et al.*, 2010). In such cases, it would be an advantage for females to mask their sexual identity in trails to reduce mating frequency (Johannesson *et al.*, 2010). How females could do this is not clear, but possibly they do so by not producing a mucus-based cue that is used by females of other species to attract males through trail-following. Indeed, the selection of trails of *L. fabalis* females over trails of *L. fabalis* males by *L. saxatilis* males indicates that the latter have retained a capacity for sex discrimination, supporting the hypothesis that trail masking is a female response (Johannesson *et al.*, 2010).

#### (e) Evolution of reproductive barriers

In many cases, cues in mucus trails can contribute to pre-zygotic barriers to mating between closely related species (see Sections II.2a and b). When the function of trail-following is to locate a suitable partner, a mechanism that prevents males following trails of females of other species would be under strong positive selection. Experimental studies in closely related species, however, show that the evolution of species-specific trail-following may be less straightforward. In the marine sister species *Littorina fabalis* and *L. obtusata*, males of the smaller *L. fabalis* prefer to follow females of the larger *L. obtusata* over females of their own species, while male *L. obtusata* do not track the smaller females of *L. fabalis* (Saltin, 2010). This suggests that, in this case, size-related trail cues may be more important than species-specific cues in closely related species, with the result that males discriminate between trails primarily on the basis of size. When males of these two littorinid species encounter females, however, heterospecific pairs interrupt copulation early, indicating the presence of a barrier to reproduction at this stage (Saltin, 2010). In the mangrove littorinid snails, *Littoraria ardouini*

and *L. melanostoma*, males of both species prefer to follow females of their own species during the mating season, but this discrimination does not occur at other times (Ng *et al.*, 2011). This temporal variation in preference suggests either variation in the quality of the females' trail and the presence of species-specific attractants during the mating season, or that males respond differently to trails between seasons. In general, male gastropods seem to have greater difficulty in distinguishing conspecific females from females of other species when the species are closely related (Trott & Dimock, 1978; Bousfield *et al.*, 1981). Hence, the evolution of trail-based, pre-zygotic barriers to mating may be impeded by close phylogenetic relationships.

Under some circumstances, trail-following may contribute to the evolution of reproductive barriers within a species. In the polymorphic intertidal littorinid, *Littorina saxatilis*, ecotypes have evolved adaptations to different shore microenvironments. In border areas between contrasting environments, ecotypes overlap and hybridize (Janson & Sundberg, 1983; Johannesson, Rolán-Alvarez & Ekendahl, 1995). Field observations show that assortative mating occurs in these areas, and laboratory tests of mating behaviour show that when females of both ecotypes are available, males of one ecotype preferentially follow mucus trails laid by females of that ecotype. The mechanism for this discrimination appears partly related to size differences between ecotypes, and partly to differences in some other unknown cue (Johannesson *et al.*, 2008). This discrimination *via* mucus trails provides a significant pre-zygotic reproductive barrier between ecotypes of this species and reduces gene flow between ecotypes by approximately 50–100% (Panova, Hollander & Johannesson, 2006). Notably, crosses of the ecotypes produce fully fertile offspring (Johannesson *et al.*, 2010) and this therefore raises the question of the role of trail discrimination in ecological speciation. One possibility is that mucus trail cues may arise after speciation in order to optimize male mate-searching strategies, and this may account for limited discrimination between closely related species, as discussed above. Alternatively, trail-based barriers to reproduction may evolve as one of the first steps in speciation, as suggested by the example of *L. saxatilis* ecotype discrimination.

### (3) Nutrition and energy conservation

#### (a) Nutritional benefit

In terms of nutrition, mucus trails appear to have two functional roles: in finding prey and as an energy source through trail ingestion. It has long been known that predatory snails follow the trails of other snails in order to locate and eat them, and this behaviour occurs across broad taxonomic groupings and habitats (Table 3). Thus laying a trail can have a survival cost to snails due to the risk of being tracked and located by both molluscan and non-molluscan predators and parasites. Non-molluscan predators include leeches and planarians, which can distinguish between trails of different snail species (Iwai, Sugiura & Chiba, 2010; Lai, Chen & Lee,

2011), and predatory sciomyzid fly larvae (McDonnell, Paine & Gormally, 2007), while parasites include haematophagous mites (Schüpbach & Baur, 2008).

It has been suggested that trail-following evolved as a means of recycling energy-rich mucus (Davies & Hawkins, 1998). While there is no direct evidence of selective mucus-trail ingestion there is ample evidence of snails feeding on particles in mucus trails during which it would be extremely likely that mucus is ingested (e.g. Davies & Beckwith, 1999; Hutchinson *et al.*, 2007). For aquatic snails, Connor & Quinn (1984) proposed that mucus trails might become organically enriched, trapping microalgae, and could subsequently be ingested by the individual that laid the trail. This strategy would function most effectively for species that follow their own trails on excursions from a central location. Indeed, it has been shown that microalgae grow better in the mucus of homing limpets (*Lottia gigantea* and *Macklintockia scabra*) than in the mucus of a non-homing limpet or a carnivorous gastropod (Connor & Quinn, 1984). Connor & Quinn (1984) also postulated that animals might add a fertilizer to their mucus to stimulate microalgal growth. According to evolutionarily stable strategy theory, animals that add a fertilizer to their mucus trails to promote the growth of food resources should also be territorial, and therefore gain a direct individual benefit from harvesting their investment; this is the case for *L. gigantea* but not for *M. scabra* (Davies *et al.*, 1992a). The trail mucus of *L. gigantea* persists from 4 to 15 days, which was argued to be sufficient to allow for algal growth and subsequent ingestion (Connor & Quinn, 1984; Connor, 1986). Davies *et al.* (1992a) recorded longer persistence times for the trail mucus of *Littorina littorea* (half-life of approximately 12 days) and for the pedal mucus produced while stationary by *Patella vulgata* (half-life of approximately 40 days). The mucus of *P. vulgata* readily collected organic material but this varied with shore exposure to wave action, with more microalgae being trapped on semi-exposed as opposed to more sheltered shores (Davies *et al.*, 1992a). The mucus of this species has also been shown to trap barnacle larvae (Holmes, 2002). The pedal mucus of some gastropods found in Chile also traps microalgal and macroalgal particles (Santelices & Bobadilla, 1996), and increased microbial activity has also been recorded in limpet and trochid mucus trails, potentially enhancing their nutritive value (Herndl & Peduzzi, 1989; Peduzzi & Herndl, 1991). On tropical shores the mucus produced while stationary by the limpet *Cellana grata* was, however, found to have a shorter persistence time (~6 days) than mucus produced by its temperate counterparts, and its capacity for organic enrichment was weak (Davies & Williams, 1995). An extreme form of organic entrapment in mucus trails is exhibited by the mud snail *Ilyanassa obsoleta*, which traps amphipods for periods of over 1 h, which are subsequently ingested (Coffin *et al.*, 2012).

All these studies demonstrate that mucus is sticky and can trap organic particles, but not that it is an important component of an animal's diet. Further studies, perhaps tracing fatty-acid signatures, are needed to confirm ingestion during trail-following as a significant mode of nutrition.

It should also be noted that in some cases the consumption of mucus might not be beneficial. Mucus produced by stationary limpets, for example, can concentrate metals from sea water by over 1000-fold (Davies & Cliffe, 2000), and bacterial enrichment of abalone mucus trails may include pathogenic species (Guo *et al.*, 2009). Nevertheless, as Davies & Hawkins (1998) highlighted, given the density of some aquatic snail populations, their movement patterns and the persistence of mucus trails, a great deal of the substratum for much of the time is likely to be covered in mucus. It is, therefore, highly likely that mucus trails will be ingested to some degree and contribute to some extent to the nutrition of benthic grazers. This supposition is further supported by the fact that on encountering mucus trails seeded with microalgae, *Littorina littorea* altered its behaviour, increasing trail-following, locomotory speed and rasping rate, and algae from the trails were ingested (Davies & Beckwith, 1999). These authors noted that 'trail following seems inextricably linked to nutrition' (Davies & Beckwith, 1999, p. 255), a viewpoint that is likely to be correct.

The functioning of mucus trails in nutrition is dependent on the production rate of mucus, which can vary substantially among species. There is a considerable body of literature describing pedal mucus production rates, but most do not measure trail mucus alone but either involve artificial stimulation of the foot or combine trail mucus with mucus produced by stationary animals (see Davies & Hawkins, 1998, for review). Those that do report on trail mucus production often express values per unit time, irrespective of how active the animals have been (e.g. Kideys & Hartnoll, 1991; Navarro & Torrijos, 1995). Although Edwards & Welsh (1982) reported trail mucus production by *Ilyanassa obsoleta* as 21.8 µg ash-free dry mass cm<sup>-2</sup> (a measure related to the area of mucus produced), a more common but less informative measure (because the width of the trail is not accounted for) is mass of dry mucus per unit distance travelled; typical values are in the order of µg mm<sup>-1</sup> (Davies *et al.*, 1992b; Davies & Williams, 1995, 1997; Donovan & Carefoot, 1997; Lee & Davies, 2000; Hutchinson *et al.*, 2007). Pedal mucus production rates are also affected by environmental conditions. Production rates by *Littorina littorea* in sea water were, for example, approximately 35% less than in air (Davies *et al.*, 1992b); and similar significant differences were also recorded for *Patella vulgata* (Davies *et al.*, 1990a) and *Monodonta labio* (Hutchinson *et al.*, 2007). This effect might be explained by the decreased apparent weight of snails in water, resulting in a reduced need for mucus to aid locomotion in relatively 'lighter' animals (Davies *et al.*, 1990a, 1992b).

#### (b) Energy conservation

Despite the potential for gaining energy through mucus ingestion, laying trails remains energetically costly, and gastropods would benefit from finding a mechanism to recoup these costs. Re-using previously laid trails, for example, can save energy in two ways. First, trail-following can be metabolically more efficient than trail-laying:



the locomotory force applied by *Littorina irrorata* was reduced while trail-following in comparison to trail-laying (Tankersley, 1989). Second, and probably more importantly, Davies & Blackwell (2007) discovered that while following fresh, conspecific, mucus trails, *L. littorea* produced only approximately 27% of the volume of mucus produced by the trail layer, and therefore expended less energy. The more the original trail decayed, the more mucus was produced by the trail-tracking snails, effectively maintaining the cross-sectional profile of a fresh trail. Trail-following, therefore, may have evolved in part as an energy-saving mechanism, providing, of course, that the tracking animal ‘wants’ to go in the same direction (Davies & Blackwell, 2007). This also has implications in terms of population self-organization and the potential evolution of ‘cheats’ in what appear to be co-operative systems (see Stafford, Davies & Williams, 2012a and Section II.4). The fact that trail-following snails still produce mucus, even when following freshly laid trails, implies that these snails are not able to stop pedal mucus production. The maximal energetic benefit of trail-following in terms of minimizing mucus production will occur on freshly laid trails; this might explain why fresh trails are followed more often than older trails (Chapman, 1998; Edwards & Davies, 2002). A similar strategy of reduction in mucus production when trail-following occurs in *Monodonta labio* on tropical shores (Hutchinson *et al.*, 2007); trails that had been followed were thinner than trails that were not followed, a phenomenon that has yet to be explained, but may involve ingestion of the trails.

#### (4) Self-organisation and aggregation

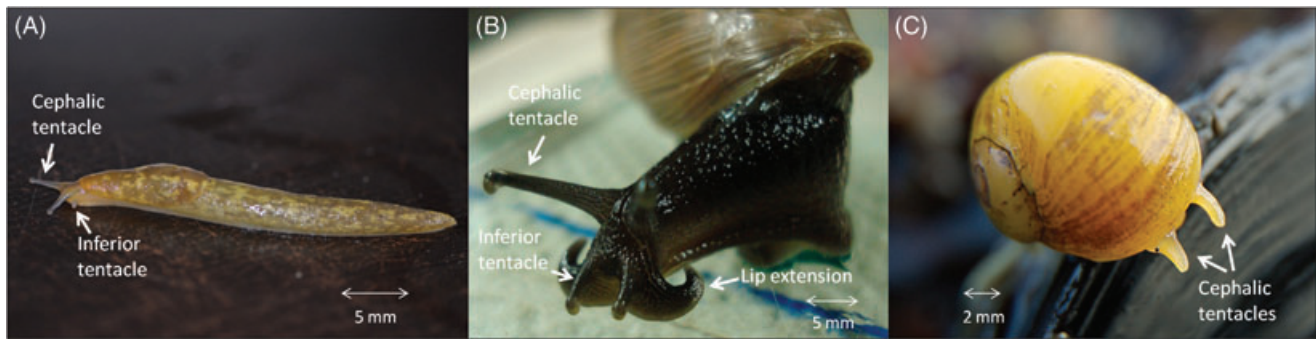
Logically, conspecific trail-following will lead to clumped, or contagious, distributions and result in aggregations, suggestive of a degree of self-organisation. This self-organisation, as described below, may be advantageous, as might the formation of aggregations themselves, which may offer a degree of protection from predation or extremes of physical environment (Stafford, Davies & Williams, 2012b). Self-organisation occurs when many parts of a system work independently, with no central coordination, but which nevertheless result in an emergent property arising at the system level (Kauffman, 1993). In the context of individuals in a population, self-organisation implies a population-level organisation through individuals interacting with each other at a local scale, and has been extensively studied in social insects such as ants. Impressive displays of organisation, such as hundreds of individuals moving in ‘single file’, have meant that research into trail-following in social insects has focussed on how these self-organised processes occur (Bonabeau, Dorigo & Theraulaz, 1999).

Patterns of self-organisation in gastropods are not perhaps as obvious as in social insects. Many intertidal species, including representatives of the Littorinidae (Chapman & Underwood, 1996; Stafford, 2002), Planaxidae (Mohammed, 1999), Neritidae (Chelazzi *et al.*, 1983, 1985) and predatory Muricidae (Crothers, 1985; Tong, 1988; Johnson *et al.*, 1998) do form dense aggregations during emersion periods.

Such aggregations are traditionally considered to function as shelters from desiccation stress, although in some cases they may also play an important role in reducing predation risk (Garritty, 1984; Chapman & Underwood, 1996; Coleman *et al.*, 1999; Stafford, 2002; Muñoz *et al.*, 2008; Stafford *et al.*, 2012a). For the nerites (e.g. *Nerita textilis*, Chelazzi *et al.*, 1983, 1985) and three species of the Littorinidae [*Melarhaphé neritoides* in the UK (Stafford & Davies, 2005) and *Echinolittorina malaccana* and *E. radiata* in Hong Kong (Stafford, Davies & Williams, 2007)], trail-following has been shown to be vital to the formation of aggregations. In computer simulations, the same rule that applies to the social insects (that of following the ‘biggest’ trail, in this case specified as that already followed the most frequently), is a requirement to mimic aggregation patterns found on real shores (Stafford *et al.*, 2007). Unlike the rapidly decaying pheromone trails of ants, however, the persistence of mucus trails over at least one tidal cycle (see Davies *et al.*, 1992a; Davies, Jones & Hawkins, 1992c; Davies & Williams, 1995, for validation of this assumption) is predicted by computer simulations to be important in maintaining the spatial positions of aggregations found on the shore over successive days (Stafford *et al.*, 2007).

Recent work demonstrates that trail-following and the temporal persistence of trails maintain aggregations, despite differences in the foraging periods of individuals (Stafford, Williams & Davies, 2011). This means that plasticity in behaviour at the individual level has little effect on aggregation levels (Stafford *et al.*, 2011, 2012a). In any biological system, this is likely to lead to the evolution of ‘cheats’ (reviewed by Nowak, 2006), individuals that attempt to exploit this plasticity in individual behaviour by maximising their foraging periods while still maintaining optimal positions inside aggregations (Stafford *et al.*, 2012a). On the high shore in Hong Kong, cheats do seem to occur, but not among individuals of one snail species, rather the cheats in this case are members of a different species. Two species of snail forage in the high shore, following the rising tides. *Echinolittorina radiata* stops foraging and moves down the shore first as the tide retreats, and begins the process of forming aggregations. The other species, *E. malaccana*, moves down the shore considerably later, following the mucus trails laid by *E. radiata* and therefore locates the same aggregations and receives the same benefits from joining those aggregations as the earlier-arriving species (Stafford *et al.*, 2012a).

Following mucus trails, therefore, allows self-organisation in intertidal littorinids, with many similarities to self-organisation processes in social insects. However, unlike in social insects, where small changes in individual-level behaviour can cause large changes in emergent patterns at the population level (e.g. Solé *et al.*, 2000), the aggregation process in littorinids appears relatively robust, probably due to the long-term persistence of mucus trails in the environment (Stafford *et al.*, 2011). Hence the nature of the trail has an influence on the plasticity of the self-organising behaviour. For high-shore littorinids, trail-following allows



**Fig. 1.** Photographs of a terrestrial slug (A) *Limax pseudoflavus*, courtesy of Anthony Cook, a terrestrial snail (B) *Euglandina rosea*, courtesy of Brenden Holland and a marine snail (C) *Littorina fabalis*, courtesy of Patrik Larsson illustrating the major sensory apparatus (arrowed) involved in the detection of mucus trails.

optimisation of the trade-off between foraging duration and time taken to find shelter.

### III. MECHANISMS OF TRAIL-FOLLOWING

#### (1) Sensory apparatus for trail detection

Most terrestrial pulmonate gastropods have two pairs of tentacles (see Fig. 1A, B): the posterior (= cephalic) and anterior (= inferior) tentacles, both of which may be involved in trail detection (Chase & Croll, 1981; Cook, 1985*b*, Chase, 1986; Davis, 2007). Through tentacle amputation experiments, Chase & Croll (1981) demonstrated that *Achatina fulica* used the anterior tentacles to detect mucus trails, whereas Cook (1985*b*) showed that *Limax pseudoflavus* (Fig. 1A) used both the posterior and anterior tentacles to follow trails. For littorinid snails, which only have one pair of cephalic tentacles (e.g. *Littorina fabalis*, Fig. 1C), frequent contact between the tentacles and the substratum has been observed during trail-following, suggesting that the tentacles play an important role during trail-tracking (Peters, 1964; Hall, 1972; Erlandsson & Kostylev, 1995; Ng *et al.*, 2011). Tentacles, however, are not the only sensory apparatus that can be used to detect mucus trails. In the terrestrial predatory snail, *Euglandina rosea* (Fig. 1B), for example, lesion of the tentacles had little influence on trail-following but removal of the buccal lip extensions strongly limited this behaviour (Cook, 1985*a*). Although no specific mechanism has been suggested, trail detection *via* the foot has also been proposed in *Navanax inermis* (Paine, 1963) and *Ilyanassa obsoleta* (Trott, 1978; Trott & Dimock, 1978).

#### (2) Cues for trail specificity

Since pedal mucus contains significant levels of protein, polysaccharide and other organic substances (reviewed by Davies & Hawkins, 1998), chemical cues have been proposed to drive trail-following in cases such as homing (Funke, 1968; Cook, 1969, 1971, 1979; Cook & Cook, 1975; Chelazzi *et al.*, 1985), conspecific aggregation (Trott, 1978; Trott & Dimock,

1978), mate-searching (Peters, 1964; Chase *et al.*, 1978; Cook, 1985*a*; Johannesson *et al.*, 2010; Ng *et al.*, 2011) and predation (Cook, 1985*a*, 1989; Marin *et al.*, 1999; Clifford *et al.*, 2003; Shaheen *et al.*, 2005). In an early study, Sleeper & Fenical (1977) reported a yellow hydrophobic substance (containing three methyl ketones) released in mucus trails of the sea slug *Navanax inermis* following its disturbance. This substance seemed to act as an alarm pheromone, since its presence in the trail deterred trail-following by conspecifics. A recent study demonstrated the presence of gamma-aminobutyric acid (GABA) in pedal mucus of abalone (*Haliotis* spp.), which facilitated larval settlement (Laimek *et al.*, 2008).

Although some species-specific chemical substances, such as proteins, have been reported in mucus (Cottrell *et al.*, 1993; Smith, 2002, 2006; Li & Graham, 2007), these studies primarily focussed on the adhesive properties of mucus. For example, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed significant differences in mucin composition between the trail mucus of seven terrestrial gastropods (Cottrell *et al.*, 1993), which may imply differences in function. Spectrophotometric techniques (based on bond and functional group presence) have been applied to determine the chemicals in both mucus trails (Lincoln, Simpson & Keddie, 2004) and mucus harvested from the foot, and have revealed interspecific differences in a variety of pulmonate and prosobranch gastropods (Skingsley, White & Weston, 2000; Lim & Tan, 2008). There are, however, few data to indicate whether these species-specific chemical cues have an ecological role, such as in trail-following behaviour. Intraspecific variation has also not been investigated to any great degree; reported differences might reflect individual-level environmental responses rather than differences among species (see Skingsley *et al.*, 2000). Many studies report on mucus that has been harvested from the foot by mechanical stimulation (e.g. Davies *et al.*, 1990*a*), or make no distinction between the mucus produced while animals are stationary or produced as a trail (e.g. Peck, Prothero-Thomas & Hough, 1993) even though these can differ considerably in their chemical composition (Iwasaki, 1992; Smith & Morin, 2002; Kuanpradit *et al.*, 2012). The composition of pedal mucus can also vary temporally, over

what may be seasonal cycles (Davies, Jones & Hawkins, 1990b), or according to the diet (Lorenzi & Martins, 2008).

The available information on chemical cues in relation to trail-following comes from two groups of studies. First, early studies found volatile low molecular weight substances in mucus, which may act as a cue to drive trail-following. These substances from the mucus trail, when diffused through a dialysis membrane, were found to trigger trail-following in the land slug *Mariaella dussumieri* (Ushadevi & Krishnamoorthy, 1980), and the freshwater snail *Biomphalaria glabrata* (Bousfield *et al.*, 1981). The substances, however, did not trigger trail-following in *Ilyanassa obsoleta* (Dunn, 1982). Second, small and highly water-soluble molecules have been implicated in trail-following. These molecules have been speculated to be small peptides or proteins because the predatory snail, *Euglandina rosea*, readily learnt to follow artificial trails composed of amino acids. Such components could quickly be dissolved out of, or rinsed from, the mucus trails of prey snails (Clifford *et al.*, 2003). Cook (1994) also showed that trail-following in the land slug *Limax pseudoflavus*, was driven by small and soluble components in the trail rather than the sticky, insoluble components of the mucus. Recently, Kuanpradit *et al.* (2012) successfully characterised three low molecular weight proteins isolated from trail mucus of the abalone, *Haliotis sinine*. These are suggested to be pheromones that diffuse from the mucus trails into the water as attractants to facilitate conspecific aggregation (Kuanpradit *et al.*, 2012). This mechanism of pheromone transmission is, however, different from that suggested for many other terrestrial and intertidal gastropods, where pheromones are mucus-bound and thought to be detected by direct contact of the tentacles with the mucus trail (Chase & Croll, 1981; Stirling & Hamilton, 1986; Erlandsson & Kostylev, 1995; Ng *et al.*, 2011).

The structural component of mucus trails may also be used as a trail-following cue. Bretz & Dimock (1983) demonstrated that structural elements were important cues and suggested that filaments in the trail were more likely to drive trail-following in *Ilyanassa obsoleta* than chemical cues. Differences in body size leading to different trail widths have also been speculated to facilitate recognition by species or ecotype in *Littorina* species (Johannesson *et al.*, 2008; Saltin, 2010, see Section II.2c). Future research, therefore, should take into account the possibility of both physical (or structural) and chemical cues in mucus trails as well as the possible use of multiple cues to drive the different functions that trail-following may serve. Without any direct evidence on the nature of trail-following cues, studies based on behavioural experiments should also be interpreted carefully, as results may depend on the experimental methods used (Cook, 2001; see Appendix for common methods used to study trail-following).

Although the specific cues that drive mucus-trail-following are yet to be identified, evidence suggests that similar cues may be evolutionarily conserved among closely related species, as the ability to discriminate between conspecific and heterospecific trails is often correlated with phylogeny

(Cook, 1977; Trott & Dimock, 1978; Bousfield *et al.*, 1981; Johannesson *et al.*, 2010; Saltin, 2010). *Limax pseudoflavus*, for example, does not follow trails laid by *Tandonia budapestensis* or *Dendrodoris reticulatum*, but will follow those of the closely related species, *Limax flavus* (Cook, 1977); a similar phenomenon has been observed in *Biomphalaria glabrata* (Townsend, 1974; Bousfield *et al.*, 1981). These responses suggest that closely related species might share the same or similar cues, confusing species recognition (see examples in Sections II.2d and e). Trott & Dimock (1978) showed that *Ilyanassa obsoleta* failed to distinguish between its own trails and those of the closely related *Nassarius vibex*, although *N. vibex* could discriminate conspecific trails from trails laid by *I. obsoleta*. This variation in discrimination may indicate that the two species have different abilities to detect the cues or that they may follow trails for different purposes.

### (3) Trail polarity and its cues

#### (a) Trail polarity

Recognition of trail polarity is obviously important when following mucus trails. When a trail is followed in the same direction in which it was laid, this has been termed following the trail 'with polarity' (Stirling & Hamilton, 1986; Robins & Hamilton, 1996; Davies & Beckwith, 1999) or 'positive polarity' (Johannesson *et al.*, 2008, 2010; Ng *et al.*, 2011). Conversely, 'against polarity' or 'negative polarity' refers to following a trail in the opposite direction to which it was laid. Species that exhibit conspecific trail-following have generally been reported to lay polarised trails [that is trails that have cues that indicate the direction in which they were laid, e.g. *Biomphalaria glabrata* (Townsend, 1974), *Dendrodoris* spp. (Nakashima, 1995), *Deroceras reticulatum* (Wareing, 1986), *Ilyanassa obsoleta* (Trott & Dimock, 1978), *Littoraria* spp. (Hall, 1972; Stirling & Hamilton, 1986; Ng *et al.*, 2011), *Littorina* spp. (Gilly & Swenson, 1978; Johannesson *et al.*, 2008, 2010), *Mesodon thyroidus* (Davis, 2007), *Nerita textilis* (Chelazzi *et al.*, 1983), *Nodilittorina unifasciata* (Chapman, 1998), *Onchidium verruculatum* (McFarlane, 1980, 1981), *Physa acuta* (Wells & Buckley, 1972)], but there are also exceptions [see *Euglandina rosea* (Cook, 1985a), *Limax* spp. (Cook, 1977, 1992), *Littorina littorea* (Edwards & Davies, 2002), *Pomacea canaliculata* (Takeichi *et al.*, 2007)].

Gastropods, in general, show positive polarity when following conspecific trails, except for some homing species where individuals often retrace their own outward-bound trails with negative polarity to return to their resting sites [e.g. *Collisella gigantea* and *Lottia scabra* (Connor, 1986), *Patella vulgata* (Funke, 1968; Cook *et al.*, 1969), *Onchidium verruculatum* (McFarlane, 1980), *Siphonaria alternata* (Cook & Cook, 1975)]. When predatory gastropods follow prey trails there is often no consistent polarity (Gonor, 1965; Cook, 1985a; Pearce & Gaertner, 1996; Gerlach, 1999). The predatory land snail, *Euglandina rosea*, for instance, showed positive polarity when following conspecific trails but failed to recognize the direction of prey trails (Cook, 1989; Clifford *et al.*, 2003; Shaheen *et al.*, 2005). By

contrast, a more recent study (Davis-Berg, 2012) found that *E. rosea* showed positive polarity when tracking prey trails. Davis-Berg (2012) attributed such contradictory findings to differences in confounding environmental conditions (see Cook, 2001). The decision to follow a trail either with, against or irrespective of polarity may be state dependent, on factors such as mating status, hunger level, or the urge to seek a refuge. Trail-following irrespective of polarity could also be explained by reasons other than location of the trail layer (e.g. nutritional benefit or energy conservation, see Section II.3). It is also likely that different species use different cues to detect polarity in mucus trails. Nevertheless, there are examples of closely related species where snails follow heterospecific individuals with positive polarity [e.g. when *Lyanassa obsoleta* follow *Nassarius vibex* (Trott & Dimock, 1978) and *Littorina saxatilis* follow *L. fabalis* (Johannesson *et al.*, 2010)], suggesting a relationship between directional cues and phylogeny.

#### (b) Cues for trail polarity

Cook (2001) summarized three possible types of directional cues in mucus trails (also see Cook, 1971; Stirling & Hamilton, 1986). First, thread-like fibres or filaments in the mucus may serve as directional cues, as has been suggested in *Lyanassa obsoleta* (Bretz & Dimock, 1983), *Littoraria irrorata* (Stirling & Hamilton, 1986) and *Littorina littorea* (Davies & Hutchinson, 1995), where the fibres are aligned linearly in the direction of the trail and are associated with calcium granules. Second, short-lived chemical cues may provide a chemical gradient along the trail as they decay successively with trail age. Such cues have been suggested in *Mariaella dussumieri* (Ushadevi & Krishnamoorthy, 1980) and *Biomphalaria glabrata* (Bousfield *et al.*, 1981). Third, chemical information may be arranged to create a left-right asymmetry of the trail. This mechanism was proposed by Shaheen *et al.* (2005), who experimentally eliminated possible structural cues and chemical gradients in mucus trails of *Euglandina rosea*. Cook (2001) further discussed the potential importance of cues external to the mucus. He suggested that animals may orientate through distant chemoreception of the cue source, which could be the trail-laying animals themselves, or cues deposited at resting sites. This mechanism, however, may be more likely to occur in homing species, where homing is often achieved through a combination of trail-following and distant chemoreception (Cook, 1969, 1971; Chase & Croll, 1981). Other external cues such as light and gravity have also been proposed, but generally have been ruled out experimentally (Cook & Cook, 1975).

While cues that drive trail-following and polarity remain unclear, one intriguing aspect is the possibility that the release or detection of cues in trails can be 'switched on' or 'off', i.e. the expression or detection of cues in trails and hence trail-following behaviour is plastic. McFarlane (1981) showed that polarity cues in the homing pulmonate, *Onchidium verruculatum*, were released in the outward trails but not during the return path. Cook & Cook (1975) also reported that trail polarity was lost after the pulmonate limpet *Siphonaria alternata* retraced its

path, which may indicate that polarity cues were altered or removed during the return journey. The release of cues may also vary temporally as Ng *et al.* (2011) demonstrated, where males of *Littoraria arduiniana* and *L. melanostoma* followed conspecific females during the mating season, but not at other times. Selective release or incorporation of cues into trails may perhaps indicate that production of cues is costly, so that gastropods only release these cues when they serve a beneficial function.

## IV. CONCLUSIONS

(1) The available evidence suggests that production of a mucus trail serves other functions to most gastropod species than simply locomotion. The deposition of a mucus trail as a gastropod moves facilitates information transfer to conspecifics, other snails, other animals and to the returning trail-layer. This information transfer could facilitate foraging patterns, navigation, nutrition, mate-searching, aggregation and other social behaviours. Mucus trails, therefore, can act as a means of intraspecific and interspecific communication.

(2) It is highly likely that exaptation (Gould & Vrba, 1982) has occurred; i.e. novel functions of trail-following have evolved rapidly, even though the original selective advantage of the trait may have been related to a different function, in this case locomotion.

(3) Generic solutions or adaptations that are favourable to any evolutionary lineage are expected to evolve repeatedly in a phylogenetic tree. The application of comprehensive gastropod phylogenies (e.g. Reid, Dyal & Williams, 2012) may provide informative insights for comparative analyses to elucidate the adaptive value and evolutionary significance of repeatedly evolving traits linked to trail-following.

(4) Understanding the genetic background of mechanisms involved in trail-following will involve identification of chemical or other cues along with their receptors and corresponding genes, as has been done for pheromone-mediated speciation in butterflies (Lassance *et al.*, 2010). Initial results beginning to link the phenotype and genotype of gastropod chemical perception are already available in the 'model' mollusc *Aplysia* (Cummins *et al.*, 2009).

(5) Future studies should consider trail-following behaviour as a holistic collective of interlinked behaviours, rather than having a single function. The main adaptive function of trail-following may vary fundamentally across different taxa, but there are striking similarities when an integrated approach is taken. For example, hydrodynamic advantages of trail-following that occur in fish probably explain the self-organisation of fish schools (Krause & Ruxton, 2002; Stafford, Davies & Williams, 2008). In ants, gradients of pheromones induce self-organised spatial patterns (e.g. Bonabeau *et al.*, 1997), while in snails, following the most frequently followed trails leads to self-organised aggregation, with attendant benefits (Stafford *et al.*, 2008, 2012*b*). In vertebrates, insects and snails different mechanisms of

trail-following may have led to the evolution of self organisation of groups of individuals.

(6) The integrated role of trail-following in gastropods may provide the basis for new hypotheses of trail-following in other motile taxa and support further investigations on the role and evolutionary background of this behaviour.

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## VII. APPENDIX: METHODS USED IN TRAIL-FOLLOWING STUDIES

One area that has caused problems in studies of trail-following across taxa is the variety of methods used to



describe and quantify this behaviour (Cook, 2001). These methods can be grouped into five classes; while we do not advocate one method over any other, we indicate the potential pitfalls and limitations of each one below.

### (1) Coincidence index

The most common method for studying trail-following is to track the movements of a 'marker' and a 'tracker' snail in an experimental arena. Typically a single marker snail is released first and allowed to move on a horizontal surface before it is removed; a tracker snail then is released on or near the trail (Townsend, 1974; Chase *et al.*, 1978; Chelazzi *et al.*, 1983; Erlandsson & Kostylev, 1995; Edwards & Davies, 2002; Hutchinson *et al.*, 2007; Ng *et al.*, 2011). The tracks of the marker and tracker snail are mapped, and the lengths of both measured as well as the length of trail overlap (i.e. the trail-following distance). A coincidence index (CI) can be calculated as:

$$CI = L_c/L_m \times L_t \text{ or } CI = L_c/L_m, \quad (A1)$$

where  $L_c$  is the length of overlapping trails,  $L_m$  is the length of the marker snail's trail and  $L_t$  is the length of the tracker snail's trail. This index varies between 0 and 1, where 1 indicates that the tracker snail followed the marker trail completely, and 0 means that there was no tracking. The CI can be used as a relative measurement of trail-following and, once appropriately transformed, can be analysed using parametric tests. While the simplicity of this method is compelling, it is however, less suitable for questions regarding choices between different trails, since snails only leave one trail and therefore only one choice is possible: to follow or not. As trackers are often placed at the starting point, facing the starting direction of the marker (e.g. Erlandsson & Kostylev, 1995; Ng *et al.*, 2011), any assessments of polarity using this system are not meaningful.

### (2) Perpendicular placement

In this method a tracker snail is placed perpendicularly, a few centimetres from an approximately straight trail produced by a marker snail. The distance and direction in which the tracker snail follows the marker trail once it encounters this trail is recorded (Clifford *et al.*, 2003; Shaheen *et al.*, 2005; Davis, 2007). This method enables the assessment of both trail-following and polarity, and is more applicable to species that display simple movement patterns (i.e. species that produce less complex trails).

### (3) T- or Y-maze

Another common method is the use of a T- or Y-maze, often used in studies of more active animals such as snakes and insects (Wilson & Hoy, 1968; Heller & Halpern, 1981; Akino, 2002), but which has also found application in studies on gastropods (Trott & Dimock, 1978; Bretz & Dimock, 1983; Nakashima, 1995; Takeichi *et al.*, 2007). A marker

snail is first released in the main arm of the Y- or T-shaped maze and allowed to crawl into either of the arms at the junction. The tracker snail is then released in the main arm and hence presented with a two-way choice. The proportion of snails following versus not following the previously laid trail is usually analysed using binomial tests. Even though the design is clear and analyses are straightforward with a simple non-parametric test, this design is restricted due to the lack of choice between alternative trails. It is also important to be aware of the risk of bias if snails adopt a certain direction due to uncontrolled experimental conditions, such as uneven illumination.

### (4) Videography and spatial mapping

This method involves video-recording several snails moving freely and interacting (e.g. Johannesson *et al.*, 2008). This provides a more natural setting for species that live in dense populations and encounter numerous trails in their environment. The arrangement provides trackers with a choice of several trails and allows researchers experimentally to address questions of preferences among different trails (e.g. sex, species and size). Allowing snails to encounter trails naturally is also suitable for studies of polarity since this approach eliminates the risk of bias from placing snails in a certain direction on a trail. Software to plot and track snails' movements is available (e.g. CellTrak for Windows, Motion Analysis Corp.; Johannesson *et al.*, 2010). Pooling the tracking results in one experimental run also provides data with a binomial distribution. Such studies are usually performed in the laboratory to provide a simple two-dimensional environment with heavy visual contrast between snails and the background to facilitate ready recognition by the software.

Studies of trail-following in the field have mostly been conducted as part of investigations into daily migrations in homing gastropods. Cook (2001) noted that the results of laboratory experiments on trail-following often deviate greatly from field observations because the former are highly simplified analogues of the natural environment. Hence, data from laboratory experiments may be misleading when trying to determine the importance of trail-following in homing. One popular method is to use time-lapse photography, which enables mapping of individual movements and trail-following in the field (Cook *et al.*, 1969; Cook, 1980). Chelazzi *et al.* (1983) attached a light-emitting diode (LED) onto each individual in a population of the nocturnal intertidal gastropod *Nerita textilis* in order to track their movements using time-lapse photography. Little *et al.* (1988; reviewed in Williams & Morritt, 1991) and Iwasaki (1998) used a different approach by marking a grid onto the rock surface; this was used to observe and to map by hand the movements of the limpets *Patella vulgata* in Ireland and *Cellana toreuma* in Japan. Davies, Edwards & Williams (2006) combined the use of LEDs and a grid in video-recordings of a population of the limpet *Cellana grata* in Hong Kong. The recording was projected at natural size onto a paper screen where positions were marked every 5 min, to allow the continuous mapping of limpets over 7 days. Previous studies using LEDs were

constrained by having only a single colour (red) of LED available; making positions of individuals difficult to resolve when they were spatially close at night. However, LEDs are now cheaply and readily available in a large colour range.

#### (5) **Computer modelling**

A novel way of studying trail-following is to model movements and trail-following *via* computerized simulations (e.g. Stafford *et al.*, 2007; Stafford, 2010). The objective

is to build a model system that resembles reality as closely as possible by incorporating data from the natural environment. By running and analysing repeated simulations many times it is possible to determine stable spatial distribution patterns and learn more about the consequences of different behaviours and their potential evolutionary origin. Even though conclusions from such models may be questioned in the sense that no real animals are involved, such an approach permits studies that could not be conducted in real life due to practical restrictions.

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