Research on sex allocation (SA) investigates how sexual organisms allocate reproductive resources to the male and female function. In “gonochorists” (terms in italic and within quotation marks can be found in the glossary in Appendix 1) SA primarily involves the maternal decision about the sex ratio of her offspring, in “sequential hermaphrodites” it concerns the timing and direction of sex change, and in “simultaneous hermaphrodites” it represents the allocation toward male and female reproductive function (e.g., the production of sperm vs. eggs). SA theory aims to predict the optimal SA that an organism should exhibit under different environmental and social conditions, which makes it a central topic in life-history theory (Charnov 1982; Stearns 1992; De Jong and Klinkhamer 2005). Given its success, SA theory is considered both “a touchstone in the study of adaptation” by W. D. Hamilton (Frank 2002), and the most successful body of evolutionary theory in predicting adaptive evolution (West et al. 2000).

A recent book synthesized our understanding of SA patterns in gonochorists (Hardy 2002). It concluded that predictions of SA theory are particularly powerful in species that can easily control SA decisions, a prime example of which are haplodiploid insects. Hermaphrodites should also have an exceptionally flexible SA (Hamilton 1967; Charnov 1982; Michiels 1998; but see West et al. 2005), because in these organisms SA simply represents a decision about how resources are allocated to different organs and behaviors within an individual. Therefore, and in contrast to gonochorists, SA will influence the immediate reproductive success of the individual rather than that of its offspring (Borgia and
Blick 1981; Michiels 1998; Michiels et al. 1999). Nevertheless, with the exception of a chapter on simultaneously hermaphroditic plants (Klinkhamer and De Jong 2002), Hardy’s book ignored hermaphroditic organisms. This is in stark contrast to Charnov (1982), who devoted similar effort to gonochorists, sequential hermaphrodites, and simultaneous hermaphrodites in his classical book. Exclusion of hermaphrodites is unfortunate, because these organisms show very variable SA patterns and drastic responses in SA to a number of environmental variables. They therefore provide excellent opportunities for an independent assessment of the factors that influence SA patterns.

The aim of this review is to summarize the theoretical and empirical advances in our understanding of SA patterns in simultaneously hermaphroditic animals, and to indicate which concepts are well supported, and where the primary gaps in our knowledge are. I restrict my coverage to these organisms for two reasons. First, the conceptual framework for understanding SA in sequential hermaphrodites is historically quite different (although, as I point out below, the distinction between sequential and simultaneous hermaphrodites is in many cases one of degree). Second, there are recent reviews of SA patterns in hermaphroditic plants (Brunet 1992; Campbell 2000; De Jong and Klinkhamer 2005), and sequentially hermaphroditic animals (Munday et al. 2006), whereas no comprehensive review of SA in simultaneously hermaphroditic animals has been published since Charnov (1982).

I have structured this review as follows. The next section discusses the distribution of simultaneous hermaphroditism in the animal kingdom, in an attempt to dispel the preconception that simultaneously hermaphroditic animals are rare. The next section introduces the main building blocks of basic SA theory for simultaneous hermaphrodites (whereas more advanced models are covered in the later sections). Section “Measuring sex allocation” deals with the difficulties of obtaining reliable measures of SA in simultaneous hermaphrodites and the kinds of data needed to improve the situation. In section “Testing for the trade-off,” I discuss a central assumption of SA theory: the trade-off between male and female allocation. I conclude that there is strikingly little supporting evidence, and indicate what kinds of studies are called for. I then examine the factors thought to limit the fitness returns for allocation to male (section “Saturation male fitness gain curves”) and female (section “Saturation female fitness gain curves”) reproduction, and discuss how the combined effects of these factors can interact with body size and the reproductive resource budget to yield size-dependent sex allocation (section “Effects of size and budget: size-dependent sex allocation”). In section “Conflicts over optimal sex allocation,” I discuss potential conflicts over SA, namely, between the nuclear and cytoplasmic genomes, between the mating partners and between hosts and their symbionts. I argue that some of these conflicts are probably unique to simultaneously hermaphroditic animals, and that they may offer interesting experimental tools to study more quantitative aspects of SA. I end the review with an outlook section into three topics that are likely to occupy us in the future, namely, the role of SA for the evolution of mating roles and sexual selection, the investigation of the evolution of SA in a phylogenetic context, and the implications of SA variation for the cost of sex in hermaphrodites.

Simultaneous Hermaphroditism among Animals

Most zoologists work on gonochorists (i.e., insects, birds, and mammals), and often consider hermaphroditic animals an oddity. A recent study has estimated that indeed only 5–6% of the known animal species (about 65,000) are hermaphrodites (Jarne and Auld 2006). This low percentage, however, raises to about 30% when excluding the extremely species-rich insects, which (interestingly) are never hermaphroditic (the example mentioned by Ghiselin [1969] appears based on flawed evidence [Disney 1993], and another example represents highly unusual transvarial transmission of sperm, rather than true hermaphroditism [Normark 2003]). Moreover, hermaphroditism occurs in 24 of the 34 animal phyla (Table 1 in Jarne and Auld 2006), being frequent or dominant in 14 (see also Table 7.1 in Michiels 1998). Furthermore, a recent paper by Eppley and Jesson (2008) shows the phylogenetic distribution of simultaneous hermaphroditism among animals, by plotting the sexual system on a phylogeny of the multicellular eukaryotes. Together, these studies clearly document that hermaphroditism is a very important reproductive mode also among animals.

Simultaneous hermaphroditism is classically associated with low population densities, and sessile, sluggish, or parasitic life styles, as its occurrence is traditionally attributed to the possibility of self-fertilization in the absence of partners and the potential of every encountered individual as mating partner (e.g., Darwin 1876; Altenburg 1934; Tomlinson 1966; Ghiselin 1969; Borgia and Blick 1981; Manning 1983). A recent theoretical study showed that high costs for mate searching can indeed stabilize simultaneous hermaphroditism in animals (Puurtinen and Kaitala 2002). Eppley and Jesson (2008) tested this idea, and found a correlation between simultaneous hermaphroditism and the costs of mate searching within a taxon among multicellular eukaryotes (although no analysis for animals alone was presented and “protandrous simultaneous hermaphrodites” were not considered to be simultaneous hermaphrodites).

Yet although density and mobility are probably important aspects, the actual phylogenetic distribution of simultaneous hermaphroditism is more complex. Shifts between “gonochorism” and simultaneous hermaphroditism are frequent in
some phyla (e.g., among the rather sessile cnidarians, and the mobile annelids and molluscs, Ghiselin 1969; Clark 1978b), whereas strong phylogenetic constraints seem to be present in others. For example, simultaneous hermaphroditism is absent in the parasitic acanthocephalans and in the frequently parasitic nematodes (nematodes like Caenorhabditis elegans are often called simultaneous hermaphrodites, but are in fact self-fertilizing sequential hermaphrodites, in which early produced sperm remain in the genital tract and fertilize eggs produced after the individual has irreversibly changed sex [Clark 1978b]). In contrast, gonochorism among the (primitively free-living and mobile) flatworms is rare (<1% among 22,500 species and fewer than ten independent origins). Finally, gonochorism seems absent in the very mobile, planktonic chaetognaths, despite many species commonly occurring at high densities (cf. Clark 1978b).

These phylogenetic patterns can partly be due to conserved developmental processes involved in the sex determination systems within these clades (Uller et al. 2007). For example, maintaining functional male and female organs within the same organism at the same time may not be possible if sex determination is based on circulating hormones, which cause the entire organism to function in one sex (a condition that is present in many gonochorists). In contrast, fairly localized patterns of signaling molecules are probably needed to regulate male and female organs in simultaneous hermaphrodites. Moreover, circulating hormones may be dangerous in hermaphrodites, as they might allow manipulation of the SA by the partner (discussed in section “Conflicts over optimal sex allocation”). A better understanding of the processes of sex determination and regulation of SA will reveal if developmental constraints influence, not only the flexibility of SA within particular sexual systems (West et al. 2005), but also their phylogenetic distribution.

The first aim of SA theory is to predict which of the basic reproductive modes, gonochorism, sequential hermaphroditism, or simultaneous hermaphroditism, a species should exhibit. The current consensus is that organisms are gonochorists if it pays individuals to specialize on one sexual role, sequential hermaphrodites if one sex has a size advantage in reproduction, and simultaneous hermaphrodites if it is difficult or expensive to find mating partners or if there are other factors that limit the fitness returns for investment in a sex function (Charnov 1982). However, given the aforementioned phylogenetic patterns, it appears that SA theory cannot yet fully explain the distribution of reproductive modes among animals. The second aim of SA theory is to predict quantitative variation in the SA patterns exhibited by an organism given its reproductive mode, and given certain environmental and social conditions in which the organism lives. It is this second aim that I will concentrate on in the following.

Basic Sex Allocation Theory for Simultaneous Hermaphrodites

The building blocks of the basic SA models for simultaneous hermaphrodites are based on a framework introduced by Charnov (1979, 1980, 1982) and Fischer (1981). These models generally make several simplifying assumptions: (1) all individuals in a population have the same fixed reproductive resource budget, and this budget is separate from the other life-history traits (Fig. 1A); (2) there is a linear trade-off between the allocation to male and female function, so that higher allocation to one function leads to a proportional decrease in the allocation to the other function (Fig. 1A); (3) fitness gain curves, which relate the investment into a sex function to the fitness that can be gained from that investment, can be described by power functions (Fig. 1B–E); and (4) generations are synchronous and nonoverlapping.

Those are evidently strong assumptions, and the basic models may therefore lack some biological realism. Indeed, as I discuss later, the evidence for the SA trade-off is limited, resource budgets can be quite variable within populations, the processes that influence the shapes of the fitness gain curves can be rather complex (thus deviating from simple power functions), and generations are often overlapping. Nevertheless, these basic models are an important starting point for SA theory and, as I discuss below, they correctly predict a number of SA patterns at least qualitatively (Fig. 1). More advanced models have been devised, which relax some of these strong assumptions, and I will also discuss them in the following sections (however, many of these more advanced models are still waiting to be tested empirically).

Interestingly, most SA models represent evolutionary models, which predict a fixed SA for all individuals within a population. However, SA can be adjusted not only in evolutionary terms, where fixed or average conditions encountered in subsequent generations select for the optimal SA (Charnov 1986), but also in terms of phenotypic plasticity, where individuals make short-term adjustments in SA in response to current conditions. Experimental tests have so far only explored cases in which SA is adjusted via phenotypic plasticity, rather than investigating an evolutionary response. I discuss some implications of this below, but in general patterns of phenotypic plasticity in SA often appear to correspond well with the predictions derived from the evolutionary models. It is, however, important to keep in mind that the two types of models are not necessarily equivalent, and that costs of phenotypic plasticity may have to be considered (Pigliucci 2005).

To test SA models one needs to be able to measure resource allocation to male and female function, to test if the trade-off assumption is justified (or to otherwise adjust the models), and to measure, or at least make informed guesses about, the processes that shape the male and female fitness gain curves (i.e.,
sex allocation trade-off

A

<table>
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<tr>
<th>reproductive resources</th>
<th>other life history traits</th>
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<td>male (m)</td>
<td>female (1-m)</td>
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<td>trait 1</td>
<td>trait 2</td>
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fitness gain curves

B

C

D

E

Figure 1. Representations of the main building blocks of basic sex allocation theory for simultaneous hermaphrodites. (A) Models generally assume a fixed reproductive resource budget that is isolated from other life-history traits (shaded areas) and a linear trade-off between male and female allocation (double-headed arrow). (B) and (C) represent two commonly used, and essentially equivalent, graphical metaphors to depict the optimization of sex allocation. Both use power functions to represent the fitness gain curves, which relate the investment into a sex function to the fitness that can be gained from that investment. In both (B) and (C) the exponent of the male power function is less than one (leading to a male fitness gain curve with diminishing returns), and the exponent for the female power function is one (leading to a linear female fitness gain curve). In (B) the emphasis is on marginal returns for investment, which in the depicted example are initially higher for the male function, whereas in (C) the emphasis is on the summed fitness of both functions. The optimal sex allocation is female biased in both examples (i.e., \( m < f \)), a scenario that is probably common in simultaneous hermaphrodites. (D) depicts the assumed effects of variation in mating group size on the exponent of the power function, and thus the shape of the male fitness gain curve. In small mating groups the exponent of the power function is smaller, and the curve therefore saturates more quickly, eventually leading to a more balanced sex allocation with increasing mating group size. Although these shape changes have rarely been measured, there is now good qualitative empirical support for an influence mating group size on sex allocation. (E) depicts the assumed effect of a limited brood space on the shape of the female fitness gain curve. Very little empirical data exist to understand how resource allocation translates into fitness). I discuss all of these issues in the following sections and refer to many empirical studies that test different aspects of SA theory (a comprehensive summary of over 50 empirical studies is presented in Appendix 2).

Measuring Sex Allocation

Besides a taxonomic bias in the types of organisms zoologists study, one reason why there are more data on SA in gonochorists is that it is relatively easy to measure SA in these organisms, as it often only requires to determine the offspring sex ratio. A potential complication arises, however, if the costs of producing a son and a daughter are unequal (e.g., Boomsma and Nachman 2002; Foster and Burley 2007). Whereas early ideas about sex ratio evolution only considered the number of male and female offspring (Darwin 1871; Dusing 1884), it was Fisher (1930) who realized that it is the total reproductive expenditure that natural selection is expected to select on (summarized by Seger and Stubblefield 2002).

Estimating SA in simultaneous hermaphrodites is more difficult, because it is a priori unclear how parameters that are chosen to represent the investment in male and female function relate to actual resource allocation (see also Queller 1984). In this section, I examine the distinction between fixed and variable costs of reproduction, and discuss the problem of comparing different male and female measures of allocation. Moreover, I point out the difference between static and dynamic measures of allocation, and stress that studies should always attempt to simultaneously obtain measures of both male and female allocation.

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EVOLUTION JUNE 2009

FIXED VERSUS VARIABLE COSTS AND STATIC VERSUS DYNAMIC MEASURES OF INVESTMENT

Several authors have suggested that it may be useful to distinguish fixed and variable reproductive costs. Reproduction requires the production and maintenance of primary sexual characters, such as genitalia, which are often considered to represent fixed (fertility-independent) costs, which have to be paid by all individuals in a population to be able to reproduce at all (Heath 1977; Charnov 1979, 1982). Such fixed costs are therefore often not considered as part of the SA of a simultaneous hermaphrodite, although they may be important for the stability of hermaphroditism (Charnov 1979). Recent research has suggested that genital morphology may primarily evolve due to sexual selection and sexual conflict (reviewed by Hosken and Stockley 2004), and that hermaphrodites may have a high investment toward sexual conflict traits (Michiels and Koene 2006). If there is variation in this type of investment between individuals in a population, then these “fixed” costs would not actually be fixed, and they would then have to be quantified as part of the SA estimates.
Genital morphology indeed shows phenotypic plasticity in response to some environmental factors (e.g., temperature, Soto et al. 2007; rearing substrate, Andrade et al. 2005; wave exposure, Neufeld and Palmer 2008; but see House and Simmons 2007). Moreover, a recent study on the barnacle *Semibalanus balanoides* (Hoch 2008), found that these animals have shorter penises in crowded aggregations, suggesting lower “fixed” costs in more competitive situations. Experimental tests of genital plasticity due to levels of sexual competition are still lacking.

Descriptive studies did not find costs of the male genitalia in species with male genital polymorphisms (also called phallic polymorphism, see Appendix S2A). Charnov (1982) has argued that, even for barnacles, which have very large genitalia relative to the body size (Neufeld and Palmer 2008), “fixed” costs could be quite small compared to the amount of reproductive energy that an animal will expend over its lifetime. The only data that bear on this suggest that the barnacle penis is actually small and degenerate outside the mating season, and regrows rapidly immediately prior to the mating season (Hoch 2008). The author speculated that this could be due to maintenance or opportunity costs arising from the space used by this large genital. Experimental prevention of the formation of the genitalia (or forcing genital regeneration in species with this ability) may allow quantifying the costs involved in building these structures.

Most SA studies have focused on quantifying variable (fertility-dependent) reproductive costs, such as resource allocation toward the production of gametes. Generally, the size (e.g., dry weight or volume) of the gonads is considered indicative of resource allocation toward a function, although that underlying assumption is rarely tested (but see, e.g., SchärER et al. 2004). Very different measures have been used to estimate gonad sizes (see Appendix 2), and their suitability depends on the study species and the structure of the gonad. If gonads are dispersed throughout the body or if male and female gonadal structures are intermingled (such as in the case of an ovotestis), obtaining dry weight estimates may be difficult and one may have to resort to quantitative histology (also called stereology, Howard and Reed 1998). Stereology is based on a systematic random sampling approach that allows to get unbiased estimates of the volumes of structures of interest from histological sections (e.g., SchärER et al. 2001).

A serious problem in estimating SA is that one unit of testicular tissue and ovarian tissue may often require different resource investments, and these may therefore not be comparable resource currencies. This compromises firm statements about the absolute level of allocation to male and female function. In plants measures of resource allocation other than dry weight or volume have been obtained in some cases (such as caloric content or elemental analysis of N, P, K, Mg, and Ca), and SA estimates based on these different measures appeared to yield fairly similar estimates (e.g., in grasses, McKone 1987). There is a clear need for such data for simultaneously hermaphroditic animals (see SchärER and Robertson 1999 for a sequential hermaphroditism). Moreover, in many organisms the variable part of male and female investment is not restricted to gamete production. It may involve investments toward the production of seminal fluids, love darts, and eggshells, or toward the performance of sex-specific reproductive behaviors, such as mate searching, courtship, or egg laying. These components should ideally also be quantified, further complicating the problem of incomparable resource currencies.

Another crucial issue when measuring variable costs is to distinguish between static and dynamic measures of allocation. The energetically costly process is the actual production of sperm, eggs, or secretion products (i.e., the energy flow through the reproductive infrastructure), but this dynamic process is often difficult to measure. Researchers therefore usually measure the size of the static infrastructure (e.g., testis or ovary size), and usually just assume that it is well correlated to resource allocation or gamete production (but see, e.g., SchärER et al. 2004; Bjork et al. 2007; SchärER and Vizoso 2007; Ramm and Stockley 2009). Such problems are particularly acute for “intermediate storage” structures that contain cells or fluids to be transferred to a mating partner, such as seminal vesicles (i.e., structures that contain sperm) or prostate glands (i.e., structures that contain seminal fluids). The distinction between static and dynamic measures of allocation are discussed in detail elsewhere (SchärER et al. 2004; SchärER and Vizoso 2007), here I only point out that it is crucial to evaluate the assumptions behind any allocation measure for a given study species.

**MEASURE BOTH MALE AND FEMALE FUNCTION**

In many organisms it is easier to study either the male or the female components of SA. This sometimes tempts researchers into just measuring the sex function that is easier to quantify and to extrapolate to the other sex function from that. For example, in many systems egg production is considerably easier to quantify than sperm production, and it is then assumed that a change in the pattern of egg production represents a SA response. This is problematic, because many models suggest that SA changes in response to variation in the intensity of male–male competition, and changes in female allocation will thus often result as a correlated response to the adjustments in male allocation. In such cases, measuring female allocation only to infer changes in male allocation can be very misleading, because it simply assumes that the SA trade-off is present (I discuss such an example in the next section). Moreover, female allocation may change for reasons unlinked to SA (e.g., due to a shift in investment between current and future reproduction). Thus without measuring both male and female allocation it can be problematic to interpret a shift in either male or female allocation alone as a SA response.
Even if both male and female allocation are measured, the way in which data are presented can still be misleading, for example, if researchers only report a combined SA measure, such as testis weight / (testis weight + ovary weight). A combined measure can vary due to variation in just one of its components, in both components, or in the worst case, it may be invariant if male and female allocation vary jointly in the same direction. Therefore, researchers should report separately the estimates for male and female allocation, and restrict themselves to report combined measures only where justified, that is, if there is evidence for the SA trade-off in the study organism (see below), or if there is little scope for trade-offs to occur between current and future reproduction (e.g., if the organism is semelparous or has a short reproductive life span).

Obtaining absolute measures of male and female allocation is probably difficult in most simultaneous hermaphrodites, and it will therefore often be impossible to say if an individual has, in absolute terms, a male- or female-biased SA. However, the situation is not as grim as this may suggest. For example, when the difference between the amount of male versus female allocation is sufficiently large, when they can both be measured in a similar resource currency (say gonad dry weight or volume), and when reproductive behaviors are not very pronounced or can be clearly attributed to the male or female function, a firm statement about an absolute bias in SA may still be possible (e.g., female-biased SA: Fischer 1981;SELLA 1990;OLIVER 1997;LOCHER and Baur 2000;SCHÄRER et al. 2001;BAEZA 2007b;male-biased SA: Hall and Hughes 1996). But even in cases in which this is not possible, relative shifts in allocation can still be studied, because it is unlikely that the costs for one unit of gonad size change drastically with the size of the gonad. In many situations one can therefore conclude, for example, that the SA of one individual in a population is more female-biased than that of another individual. These kinds of shifts are much easier to measure, and this is what the majority of the studies have done (see Appendix 2).

A final caution concerns the usage of male reproductive success as a measure of male allocation (e.g., Lorenzi et al. 2006, 2008). This is problematic, because it simply assumes a direct link between male allocation and male reproductive success (i.e., it assumes a linear male fitness gain curve). An important aim of SA research is to measure this relationship, not to just assume it.

**Testing for the Trade-Off**

Despite the fact that a linear trade-off between male and female allocation forms the basis of almost all SA models, there is strikingly little evidence for it from studies on animals (and a similar conclusion was reached in a recent review on plants, Campbell 2000). In this section, I discuss the available evidence in some detail, and suggest several experimental approaches to test the assumption.

Studies often find no correlation (e.g., Baeza 2007b) or a positive correlation (e.g., Locher and Baur 2000; Schärer et al. 2005) between male and female allocation, rather than the expected negative correlation. I am aware of only three studies that have found supporting evidence for the SA trade-off in animals (DeVisser et al. 1994; Yund et al. 1997; Schärer et al. 2005), and only in one of these was the trade-off very clear (DeVisser et al. 1994, but see below). This weak support can be interpreted in two ways. One is that the trade-off is present, but that methodological issues make it difficult to demonstrate it; the other may suggest that the trade-off assumption is actually flawed.

On the methodological side it has been suggested that phenotypic life-history trade-offs are notoriously difficult to measure, because they can be obscured by variation in individual resource budgets (vanNoordwijk and De Jong 1986). Schärer et al. (2005) have discussed this in detail in the context of the SA trade-off, and I therefore do not spend much time on this here. In some cases it may be sufficient to control for budget differences by including individual size in the analysis (a parameter likely to be strongly correlated with the resource budget). Or it may be required to grow animals under highly controlled conditions and to maybe even reduce genetic variation in resource uptake and conversion ability, while at the same time trying to induce variation in SA (e.g., Schärer et al. 2005). It is worthwhile to point out that it is probably easier to demonstrate a SA trade-off in species with a balanced SA than in those with a very biased SA. If, for instance, only about 5% of the total allocation goes to the male function then even very small budget effects can obscure the SA trade-off.

Another approach is to experimentally manipulate resource allocation, by preventing the formation of (or investment toward) the male reproductive function and to look for an increase in the output in the female function (or vice versa). For example, in the freshwater snail *Lymnaea stagnalis* the experimental cutting of a particular nerve, which connects the prostate gland with the brain, completely stops male reproductive activity (De Boer et al. 1997), and it was suggested that this leads to a reallocation of reproductive resources from the male to the female function. In an elegant study, DeVisser et al. (1994) showed that cutting this nerve indeed leads to an almost exact doubling of the number of eggs produced by treated snails compared to control and sham-operated snails (see Koene 2006 for a reanalysis of these data, which treats control and sham-operated snails separately). This result has until recently been considered the clearest experimental support for the SA trade-off in simultaneously hermaphroditic animals, and it suggested that allocation to male and female function was about equal in paired *L. stagnalis*. 

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**PERSPECTIVE**
However, egg number is only a reliable measure of female allocation if the investment per egg does not change in response to the treatment. Recent careful studies using different mating regimes have now clearly shown that there exists a trade-off between egg number and the investment per egg (measured as the clutch dry weight per egg), and that this trade-off depends on the number of mating opportunities: many eggs with a small investment per egg are laid without mating (or with low mating rates) and fewer eggs with a larger investment per egg are laid with high mating rates (J. M. Koene, pers. comm.). Moreover, it has been shown that the prostate gland secretions, which these snails transfer along with the sperm during mating, are at least partly responsible for this shift (J. M. Koene, pers. comm.).

The shift toward higher egg numbers in the treated snails in the De Visser et al. (1994) study may thus stem from the fact that they no longer received the prostate gland secretions (because they were held with other treated snails, and therefore did not mate), whereas both the control and sham-operated snails still mated normally, and thus received these secretions. Contrary to the claims of De Visser et al. (1994) there may have been no overall change in female allocation in response to the treatment (J. M. Koene, pers. comm.). This means that the original interpretation, that is, that the reported change in the number of eggs produced represents a shift in SA, is probably flawed. Quantification of male allocation would likely have prevented this faulty interpretation, underlining the points I made above.

A novel but conceptually similar approach to test for the SA trade-off would be to knock out specific genes responsible for the formation of the male or female gonad (e.g., using RNA interference, which was recently established in the free-living flatworm Macrostomum lignano, Pfister et al. 2008). However, no data on such an approach have been reported so far.

The basis behind many phenotypic life-history trade-offs are genetic trade-offs, and these can be studied through quantitative genetics. Specific breeding designs allow to separate phenotypic variation into environmental and genetic components, and these arguably represent the most powerful way to identify genetic trade-offs (Roff 2000). However, I am aware of only one study in which quantitative genetics has been used to study the SA trade-off in simultaneously hermaphroditic animals. Yund et al. (1997) showed a trade-off between male and female allocation in the colonial ascidian Botryllus schlosseri. However, the picture was complicated by the fact that there appeared to be a three-way trade-off with asexual growth rate, rather than a clear SA trade-off. Finally, another unexplored approach could be direct artificial selection on, for example, sperm production rate, which, assuming a SA trade-off, would be expected to select for an opposing shift in female allocation.

Given the central importance of the trade-off assumption in almost all SA models, an evaluation of its validity in many more study species is essential. Importantly, the SA trade-off need not be general. For instance, it has been argued that in many plants the assumption of a direct SA trade-off is questionable because of the temporal separation of the initial investment in pollen and ovules at one time, and the provisioning of fertilized ovules at a later time (e.g., Charnov et al. 1976; Zhang and Jiang 2002; Sato 2004; but see Lloyd 1982). Similar situations may occur in animals that brood their eggs, or that otherwise provide parental care. Moreover, male and female allocation may be limited by different resource constraints, a question that was recently explored theoretically (Venable and Lloyd 2004). For example, in the land snail Arianta arbustorum the spermatophore contains more carbon and nitrogen per unit dry weight than eggs (Locher and Baur 2000), so a shortage in one of those nutrients may affect male and female allocation differently.

Importantly, low overlap in resource requirements for the male and female function is one of the classic hypotheses for the advantage of simultaneous hermaphroditism over gonochorism (Charnov et al. 1976), and so this possibility should not be dismissed without good evidence.

**Saturating Male Fitness Gain Curves**

**CLARIFYING TERMINOLOGY**

The majority of SA models for simultaneous hermaphrodites concern predictions that in some way deal with “local mate competition” (LMC), a concept that was originally formulated for gonochorists (Hamilton 1967), but which has since been extended to simultaneous hermaphrodites (Charnov 1980; Fischer 1981; Charnov 1982; Fischer 1984; Petersen 1991). Unfortunately, the term LMC is not very suitable for these organisms, being potentially misleading. In the following paragraphs, I discuss this issue and attempt to suggest a better term.

In gonochorists LMC occurs when related individuals compete with each other for access to mates (Hamilton 1967; Taylor 1981). LMC often occurs due to an ecological or a life-history constraint, such as all offspring of a mother growing up and mating within a local group, for example, within a fig in the case of fig wasps. From the perspective of the mother, competition between her sons is wasteful and she can maximize the number of grandchildren she produces by reducing the number of sons (and hence the competition between them) and increasing the number of daughters (hence providing more mates to her sons), leading to a female-biased sex ratio (Taylor 1981). With an increasing number of (generally unrelated) females laying eggs into the same fig, mate competition occurs less and less among related males, but more and more among unrelated males. As a consequence the mothers should increase their allocation to sons and the sex
ratio is expected to approach equality. There is widespread support for this scenario in gonochorists (Hardy 2002), and although LMC is often associated with inbreeding (due to brothers mating with their sisters), the sex-ratio adjustment generally occurs independently of inbreeding (Charnov 1982; but see Frank 1985; Herre 1985). Instead it represents an interaction between selection at the individual level due to competition between related individuals for mates, and selection at the level of the different “local” groups (i.e., the populations in the different figs in the fig wasp example) with respect to the different productivity of groups with different sex ratios (Taylor and Bulmer 1980; Charnov 1982).

In simultaneous hermaphrodites a similar phenomenon occurs, which also leads to female-biased SA, but which does neither require the mates to be related nor usually involves inbreeding. It arises when competition occurs between related sperm for the fertilization of ova of the mating partner (Greeff et al. 2001; Schärer and Wedekind 2001). In a monogamously mating simultaneous hermaphrodite any sperm in the sperm receiving organ of the recipient will find itself together with related sperm from the same donor (which, like brothers in gonochorists, have an average relatedness of 0.5) competing for the same set of eggs. From the perspective of the donor, it does not pay to produce more sperm than required to fertilize the available eggs of the recipient. Instead the donor can maximize the number of offspring it produces by reducing the allocation toward the production of its own eggs, by reducing the allocation toward the production of sperm, and increasing the allocation toward the production of its own eggs, leading to a female-biased SA. With an increasing number of (generally unrelated) sperm donors who deposit sperm in a recipient (i.e., with an increasing “mating group size”), “sperm competition” occurs less and less between related sperm and more and more between unrelated sperm, and so allocation to sperm will pay off in terms of increased paternity, eventually leading to a female-biased investment (Charlesworth and Charlesworth 1981; Charnov 1982).

In analogy to LMC in gonochorists I suggest to call this phenomenon “local sperm competition” (LSC), a term that nicely combines the familiar terms LMC and sperm competition, and which therefore captures well the reason for the SA adjustment (i.e., sperm competition between related sperm). Greeff et al. (2001) have previously suggested the term “sibling-gamete competition” for this phenomenon. On one hand, this term is attractive because it includes pollen, and would thus allow the same term to be used for animals and plants. But this term is also problematic (1) because it includes eggs and ovules, which are of course also gametes (and local competition between eggs or zygotes is generally referred to as a different process, see below), and (2) because the sperm (or pollen) need not actually be siblings, but it suffices if they are related.

An example for the confusion that the term LMC can cause is a recent study that presents a model on the influence of mate search efficiency on the evolution of SA and the stability of simultaneous hermaphroditism (Puurtinen and Kaitala 2002). In their paper, the authors explicitly state that their model is not an LMC model, because the “LMC model only applies to populations that are divided into a large number of isolated mating groups.” However, as outlined above, in simultaneous hermaphrodites such a population structure is not needed to produce LMC (in the sense of LSC). The shifts in SA and instability in gender expression that Puurtinen and Kaitala (2002) observe in their model at high density is simply the result of reduced LSC due to high encounter and mating rates, leading to a large mating group size under these conditions.

THE LOCAL SPERM COMPETITION PERSPECTIVE

From the LSC perspective one can regard the range from selfing or monogamy (no sperm competition) to small mating group size (weak sperm competition) to large mating group size (strong sperm competition) as a continuum from strong to intermediate to weak LSC, respectively. In simultaneous hermaphrodites LSC and sperm competition are therefore in some way the opposite of each other. LSC representing competition between related sperm and sperm competition the competition between unrelated sperm (see also Greeff et al. 2001; Schärer and Wedekind 2001).

Therefore, any process that affects the intensity of LSC is expected to affect the shape of the male fitness gain curve. Specifically, LSC will lead to a saturating male fitness gain function; after initially high returns for investment in the male function the return quickly decelerates due to LSC, and the remaining resources should be channeled into the female function. Therefore, high selfing rate and a small mating group size both lead to LSC and have the same predicted effect on SA: both should favor a female-biased investment (Charlesworth and Charlesworth 1981; Charnov 1982).

Moreover, by focusing on the interactions between sperm of the same versus different donors it becomes clear that factors other than selfing and mating group size may influence the intensity of LSC (Fig. 2). One such factor is random paternity skews (Fig. 2C), as a result of which “individuals will have very different proportions of their zygotes sired by different fathers” (Greeff et al. 2001). Such random skews could, for example, stem from stochastic effects on mate encounter rates, nonrandom spatial distribution of individuals or imperfect mixing of sperm in spormcast mating organisms. An example from (hermaphroditic)
Factors that lead to local sperm competition (LSC). Under selfing or monogamy (A) only related (same color) sperm are competing for the fertilization of the recipient's ova, and this is expected to select for a reduced allocation to the male function. In small mating groups with a fair-raffle sperm competition (B) the amount of sperm stored is in proportion to the amount of sperm produced and transferred. In contrast, random paternity skews (C), sperm displacement (D), and cryptic female choice (E) generally result in more intense LSC, because some of the sperm produced by some of the individuals do not effectively enter into the competition. This should generally select for a lower sex allocation than that expected based on the mating group size alone (mating group size is three in cases B–E).

EVIDENCE FOR SATURATING MALE FITNESS GAIN CURVES

To date, there exists only descriptive or correlational evidence for the evolution of female-biased SA patterns in species that are selfing (Johnston et al. 1998), monogamous (Fischer 1981; Sella 1990), generally have small mating groups (Fischer 1984; Petersen 1991; Locher and Baur 2000; Schärer et al. 2001; Schärer and Wedekind 2001), or are parthenogenetic and retain residual male function due to sperm-dependent parthenogenesis (Weinzierl et al. 1998; Weinzierl et al. 1999) (see also Appendix 2C). There is thus a clear need to confirm such patterns experimentally. The success of experimental evolution under enforced monogamy versus polygamy to study reproductive allocation in gonochorists (Holland and Rice 1999; Hosken and Ward 2001; Hosken et al. 2001; Pitnick et al. 2001) suggests a promising approach. In a simultaneous hermaphrodite, experimental evolution under monogamy in a usually polygamous species is expected to lead to a reduction in male allocation and an increase in female allocation, and eventually a female-biased SA.

An increasing number of studies have shown phenotypic plasticity in SA in response to variation in the mating group size (see also Appendix 2C). Some studies have only shown this on a descriptive level (e.g., Raimondi and Martin 1991), but others have experimentally manipulated social group size (e.g., Trouvé et al. 1999; Schärer and Ladurner 2003; Tan et al. 2004; Schärer et al. 2005; Brauer et al. 2007; Schärer and Vizoso 2007; but see, e.g., Schärer and Wedekind 2001; Baeza 2007a), and as a result of it the mating group size (Janicke and Schärer 2009). These studies have generally found a shift toward a higher male allocation in response to weaker LSC, as predicted in the evolutionary models.

Michiels (1998) has previously argued that a plastic or flexible SA may be one of the main advantages of simultaneous hermaphroditism over gonochorism, and the fact that a range of species adjust their SA in this way greatly supports this idea. Moreover, the presence of plasticity in SA in response to social group size suggests that variation in mating group size is an important environmental parameter for these organisms. It would therefore be interesting to obtain quantitative data on the temporal as well as spatial variation in mating group size and LSC from natural populations.
Some authors have concluded that the observed shifts in SA represent adaptive responses to the experimentally provided social environment. However, an experiment that yields a response that is in agreement with a theoretical prediction does not suffice to conclude that the response is adaptive. A study by Hughes et al. (2003) on colonies of the bryozoan *Celleporella hyalina* offers an excellent example of the care that is required before concluding the adaptive nature of a response. They showed that placing an unrelated colony next to a focal colony led to an increase in the number of male zooids produced by the focal colony, which could easily be interpreted as an adaptive shift in SA to an increase in mating group size. However, careful experiments revealed that a range of factors unrelated to mating group size can also lead to this response (e.g., a temperature shock or placing an abiotic physical obstruction close to the focal colony). Each of these adjustments could of course be a specific adaptation in response to the manipulated factor rather than a general response to a stressor, but it remains unclear if they actually are. The final step must therefore be to show that the SA adjustment in fact yields a fitness advantage under the condition in which it was produced, and to see if it can be maladaptive under other conditions.

A recent study in the polychaete *Ophryotrocha diadema* also manipulated social group size (Lorenzi et al. 2005). However, contrary to their—partly contradicting—claim, they did not actually find support for a saturating male fitness gain curve, as no shifts in allocation to sperm production were found when changing mating group size (their fig. 5). They reached this conclusion by interpreting a (drastic) decrease in egg laying (their fig. 4) as an increase in sperm production, thus illustrating the dangers of using combined SA measures (their fig. 1). It appears that sizable amounts of male allocation can go toward aggressive behavior in *O. diadema* (which may be primarily male driven, Lorenzi et al. 2006), rather than to sperm production. It would therefore be very interesting to determine the adaptiveness of aggressive behavior in this species and whether it trades off with egg production.

**THE SHAPE OF THE MALE FITNESS GAIN CURVE**

Although LSC is predicted to lead to a saturating male fitness gain curve and to a female-biased SA, an idea generally supported by the above studies, there is currently no good understanding of the actual shapes of fitness gain curves in simultaneously hermaphroditic animals. The power functions used in the basic SA models to represent the male (and female) fitness gain curves are arbitrary, and were only chosen because they allow to generate a large range of saturating and accelerating shapes and because they have convenient mathematical properties (Charnov 1979, 1982).

I am aware of only three studies that have explicitly attempted to study paternity as a function of male investment in simultaneously hermaphrodites (Appendix 2C) and to infer the shape of male fitness gain functions in different social situations (Yund and McCartney 1994; McCartney 1997; Yund 1998). Only one of these studies found evidence in support of a saturating male fitness gain curve under strong LSC (Yund 1998), whereas the other studies found linearly increasing returns with higher male investment, both with and without strong competitors. Moreover, all these studies were done on species with spermcast mating, which have been shown to have somewhat unusual sperm-usage patterns (Bishop et al. 1996; Pemberton et al. 2003).

It is unfortunate that no comparable data exist for internally fertilizing simultaneous hermaphrodites. A number of theoretical models have explored other shapes (e.g., Frank 1987; Greeff and Michiels 1999; Pen and Weissing 1999; Angeloni et al. 2002), such as sigmoid curves, which can lead to interesting SA predictions, such as male-biased SA. Sigmoid curves may occur where an initial minimal investment in the male function has to be made before any fitness can be gained. Such a scenario is plausible for internally fertilizing simultaneous hermaphrodites with sperm digestion or in cases in which sperm trading occurs.

More realistic expectations for the shape of fitness gain curves can be generated by explicitly modeling a biological mating process, which may include parameters such as mating rates and sperm displacement rules (Charnov 1996; Pen and Weissing 1999), female genital traits involved in sperm storage (Angeloni et al. 2002), random paternity skews (Greeff et al. 2001), and cryptic female choice (E. Van Velzen, L. Schärer, and I. Pen, unpubl. ms.). In sperm competition models for gonochorists, different predictions for male allocation to sperm production have been derived from models that consider mating roles and sperm precedence patterns (Parker 1990; Ball and Parker 2000) or that distinguish between the risk and the intensity of sperm competition (Parker et al. 1996, 1997). It is interesting to point out that these models have some similarity to recent models of asymmetrical LMC in gonochorists (Shuker et al. 2005), which consider how temporal patterns in egg laying and the resulting differences in LMC can influence the optimal sex ratio.

Such biological processes should be included in models for simultaneous hermaphrodites to make predictions on the shape of fitness gain curves and the resulting SA patterns. However, compared to gonochorists, there is still a fairly limited understanding of the processes of sperm competition in simultaneously hermaphroditic animals (but see, e.g., Baur 1994; Rogers and Chase 2002; Angeloni 2003; Pongratz and Michiels 2003; Evanno et al. 2005; Chase and Blanchard 2006), and quantitative data on sperm precedence patterns, sperm displacement, sperm digestion, and cryptic female choice are dearly needed.

Anyway, experimental control over male reproductive allocation is clearly required to accurately measure the shape of the
male fitness gain curve. Ideally, it should be possible to produce individuals with a range of different SA patterns, and to test their male reproductive success in different social situations. This is a difficult task in most animals, as the gonads are internal organs that are difficult to manipulate. Exceptions are colonial organisms, in which colony fragments of different sizes can be used to represent individuals with different amounts of male allocation (Yund and McCartney 1994; McCartney 1997; Yund 1998). Induction of a phenotypically plastic SA response, artificial selection experiments on sperm production rate (or testis size) or experimental knockdown of the testis could be promising approaches for manipulative experiments in other organisms. Ultimately, however, many different postcopulatory processes may lead to very similar theoretical predictions for the shape of gain curves (e.g., Pen and Weissing 1999), so ideally we should aim at disentangling their effect.

**Saturating Female Fitness Gain Curves**

Female fitness gain curves are often considered to be linear in simultaneously hermaphroditic animals, suggesting that what limits female fitness is the amount of resources that an individual can accumulate and invest in the female function. For this reason it has been suggested that Bateman’s principle applies also to these organisms (Bateman 1948; Charnov 1979). There are, however, a number of factors that may limit the returns from allocation to the female function which are not linked to pre- or postcopulatory sexual selection.

One such factor is brooding. SA models for brooding organisms usually assume linear fitness returns for allocation to the female function until the produced eggs completely fill the available brood space (Heath 1979; Charnov 1982). Once this point is reached, no further fitness can be obtained from additional female allocation, and the rest of the resources should be allocated to the male function (Fig. 1E). This is expected to lead to a male-biased SA if the brood space can be filled with less than half of the reproductive resources (Charnov 1982).

Although there is some support for an evolutionary link between brooding and simultaneous hermaphroditism (Ghiselin 1969; Premoli and Sella 1995), there are not many SA estimates of brooding species (Appendix 2D). One study reported a female-rather than a male-biased SA in a brooding asteroid (Strathmann et al. 1984), but this was thought to result from a saturating male fitness function due to partial selfing. Similarly, several brooding species of the polychaete genus *Ophryotrocha* probably show strongly female-biased SA, likely as a result of strong LSC linked to pair formation and cooperative brooding (Sella 1990; Premoli and Sella 1995). A male-biased SA was reported in a brooding coral (Hall and Hughes 1996), but the same study reported on another, nonbrooding, coral species with an equally strongly male-biased SA, which may question the link to brooding in the former. Finally, the brooding shrimp *Lysmata wurdemanni* does not appear to have a strongly limiting brood space and it actually has an extremely female-biased SA, probably because it is monogamous (Baeza 2007b). Thus evidence for brooding to be linked to a male-biased SA is currently equivocal at best (the absolute SA patterns in the brooding bryozoan *C. hyalina* and the ascidian *B. schlosseri* are not clear because of the strongly different currencies used to measure male and female allocation).

Other models predict that competition between related individuals for limiting resources, called “local resource competition” (LRC), can affect the shape of the female fitness gain curve. Like LMC, LRC was originally formulated for gonochorists (Clark 1978a), but later extended to simultaneous hermaphrodites (Charnov 1982; Lloyd 1982). It occurs if related individuals (often offspring of the same mother) compete for resources, and if the offspring derived from the female function are more clumped in space than those from the male function (Lloyd 1982; see also Schärer et al. 2001). LRC is expected to lead to a saturating female fitness function, because resource competition between maternal siblings increases with increasing female allocation, which may result in a male-biased SA. However, I am not aware of studies that have tested this hypothesis in animals. In colonial marine invertebrates with sperm cast mating, offspring derived from the male function may often be spread over a much wider range than those from the female function, which are released as advanced larvae from the maternal brood space. In such a case brooding and LRC could jointly act in the same direction, making these organisms a particularly appealing case study. An experimental evolution experiment manipulating dispersal and brooding with a simultaneous hermaphrodite would be a powerful approach.

Both theoretical and empirical research has to date focused on the male fitness gain curve, so information on female fitness gain curves is even more limited. There is a lot of scope to explore how different life-history and dispersal strategies may influence the shape of the female fitness gain curves, and how it covaries with mating group size. Whereas limiting effects of brooding can probably be studied in the laboratory (unless limitations come from, e.g., predation), the processes involved in LRC are strongly dependent on the ecological context in which the offspring grow up and will therefore require spatially explicit genotyping of the distribution of offspring produced by a maternal individual, as well as the assessment of the degree of LRC between them. This may be challenging for mobile simultaneous hermaphrodites, unless large clutches are laid and offspring do not disperse quickly after hatching. Sedentary organisms with short-range dispersal would probably be better for such studies (e.g., brooding corals, Carlon 2000).
In any case, studies will have to include manipulative experiments, such as experimental variation of brood space limitation or manipulation of the intensity of LRC. Like in the case of male fitness gain curves, the amount of female allocation should be manipulated to determine the full range of the actual shapes of the female fitness gain curve under these circumstances.

**Effects of Size and Budget: Size-Dependent Sex Allocation**

An important parameter that was ignored in the basic SA models for simultaneous hermaphrodites, and which was only recently incorporated, is individual variation in body size (Klinkhamer et al. 1997, Angeloni et al. 2002, Zhang and Jiang 2002, Cadet et al. 2004). Given that mature individuals of many species can vary in body size by an order of magnitude (e.g., Hughes and Hughes 1986; Hall and Hughes 1996; Angeloni and Bradbury 1999; Schärer et al. 2001; Anthes et al. 2006a), and that the size of the reproductive resource budget often covaries with body size, this is evidently an important inclusion. Moreover, variation in body size appears to be the single most important parameter to influence SA in sequential hermaphrodites (i.e., the size-advantage model, Ghiselin 1969), thus being likely to also influence SA in simultaneous hermaphrodites.

Consider a species with a saturating male fitness gain curve and a linear female fitness gain curve (Fig. 1B). Further assume that the reproductive resource budget varies in proportion with individual size and the shape of the fitness gain curves does not depend on the size of the individual itself. SA is optimized by investing into the function that yields the higher marginal return for each additional reproductive investment. Optimally, small individuals with few resources should thus invest first in the male function due to the initially steep increase of the male fitness gain curve. With increasing size, individuals reach the point where the fitness returns for investing in the male and female function are equal, and larger individuals should channel additional investment to the female function. Thus small individuals would be male or male-biased, large individuals would be female-biased, and SA would be size dependent (cf. Vizoso and Schärer 2007). This scenario is very similar to the strongly male-biased sex ratio that parasitic wasps produce when laying only a few eggs on an already parasitized host, and the increasingly more female biased sex ratio they produce as more eggs are laid (Werren 1980).

There is increasing evidence that size-dependent sex allocation (SDSA) is widespread, not only in plants in which it has obtained considerable attention (reviewed in Klinkhamer et al. 1997; De Jong and Klinkhamer 2005), but also in simultaneously hermaphroditic animals (e.g., St. Mary 1994; Petersen and Fischer 1996; Trouvé et al. 1999; Schärer et al. 2001; Baeza 2006; Baeza et al. 2004); there are a number of organisms that seem to show a mixture of both patterns (Berglund 1986; Petersen 1990; St. Mary 2000; Sella and Lorenzi 2003; Baeza 2006).

A recent theoretical paper has developed a model that unites sequential and simultaneous hermaphrodites (Cadet et al. 2004), and it does so by distinguishing between direct and budget effects of size. Direct size effects are linked to the competitiveness of an individual solely due to its body size, whereas budget effects are linked to the different amounts of male and female gametes an individual can produce if it has more or less resources available for reproduction. Both can have an effect on the optimal SA and both can probably vary somewhat independently (cf. Zhang and Jiang 2002). Many direct effects probably occur due to processes of precopulatory sexual selection. In a species with coercive mating or territorial defense, body size may give an advantage to large individuals over small ones in mating in the male role, thus promoting a higher male allocation in larger individuals. In contrast, budget effects are probably rather linked to processes of postcopulatory sexual selection. For example, given a certain shape of the male fitness gain function the optimal amount to be allocated to the male allocation would remain constant with increasing budget, and so the female allocation would vary with different budget sizes, leading to a budget effect on SA. Cadet et al. (2004) discuss in detail direct and budget effects from a theoretical perspective, and conclude that direct and budget effects are expected to lead to SDSA in many, if not most species (provided that there is sufficient variation in adult body size). Here I focus on empirical approaches to test for SDSA and stress that, as for other SA patterns, there is a clear need to test if SDSA is in fact adaptive.

A purely descriptive, but initially crucial approach is to sample individuals spanning the entire size range of adults in a natural population and determine their SA patterns. Care has to be taken to avoid including potentially isolated social groups of different sizes. Otherwise SA patterns may primarily reflect local adjustments to variation in mating group size, food conditions, and genetically different cohorts. An inventory of SDSA patterns among a range of different types of simultaneous hermaphrodites would be highly welcome (e.g., mobile vs. sessile, internal vs. sperm cast vs. external fertilization, copulating vs. hypodermic, unilateral vs. reciprocal mating). Especially, SA estimates for species that show pronounced size-dependent changes in gender role preferences.
would be interesting (e.g., DeWitt 1996; Angeloni and Bradbury 1999; Ohbayashi-Hodoki et al. 2004; Yusa 2008).

The next step should be to try to experimentally induce variation in body size in a genetically homogenous cohort of individuals, for example, using a controlled feeding experiment. Such an experiment could be used to collect both information about the temporal change in SA during the growth phase and to see if individuals reach a stable SA at some stage in their lives (e.g., Vizoso and Schärer 2007).

As I discussed above it is difficult to measure the shape of the fitness gain curves as a function of resource allocation. Testing the model of Cadet et al. (2004) is even more challenging, as in their model the direct effects of size and the budget effects are represented by four power function exponents (i.e., direct and budget effects on male and female fitness, respectively). Experimental tests of the shapes of these fitness gain curves would therefore require to experimentally vary size and budget independently to study their effects on fitness gains. Moreover, differences in the fitness returns for size and budget are expected to lead to complex trade-offs between growth and reproduction (Zhang and Jiang 2002), which were not explored by Cadet et al. (2004), who assumed fixed size distributions in the population. An interesting experimental approach in this context is to test if an individual that is transferred from a population in which it is small into a population in which it is large adjusts its SA and growth rate (see, St. Mary 1994 for an example in a species with strong direct effects).

Conflicts over Optimal Sex Allocation

As I have shown in the previous sections many simultaneous hermaphrodites have the ability to plastically shift their SA in response to current social or environmental conditions. This flexibility could involve considerable risks, because it may allow conflicts of interests over the optimal SA between different genetic entities, such as the nuclear and cytoplasmic genomes, the mating partners, and hosts and their parasites or mutualists. It has been argued that genetic sex determination systems in gynochorists may result from such conflicts over the sex ratio between the parents and their offspring (Beukeboom et al. 2001; Werren et al. 2002). In contrast, parent–offspring conflicts over SA are unlikely in simultaneous hermaphrodites, particularly under conditions that favor flexible SA responses to variable environments, which the offspring can presumably assess more accurately than the parents (Borgia and Blick 1981; Michiels 1998).

I have also concluded that gaining experimental control over the SA an individual exhibits is one important requirement to experimentally test SA theory. Understanding ways in which these different genetic entities affect SA may also provide us with a toolkit to experimentally fine-tune the SA of a focal individual. In this section, I outline these potential conflicts, and discuss which of them may be suitable for an experimental approach.

CYTOPLASMIC ORGANELLES

Cytoplasmic organelles, such as mitochondria (or chloroplasts in plants) are only transmitted via the female function in the majority of eukaryotes (Birky 2001). One can therefore expect a conflict of interest over the optimal SA between the nuclear DNA (nDNA) and the mitochondrial DNA (mtDNA) of an individual. The optimal SA from the viewpoint of the nDNA is as outlined in the previous sections. In contrast, the optimal SA from the perspective of the mtDNA is the complete allocation of all reproductive resources to the female function, because allocation to the male function does not enhance the transmission of the mtDNA. So a mutation in the mtDNA that reduces or inhibits the allocation to the male function will, given a SA trade-off, lead to an increased allocation to the female function and to the spread of that mutation. In agreement with this scenario, there is extensive evidence from simultaneously hermaphroditic plants that mutations in the mtDNA can cause cytoplasmic male sterility (CMS), and that CMS is often associated with increased female fecundity (Lewis 1941; Frank 1989). That “gynodioecy” in plants is often associated with resource reallocation to the female function was a phenomenon that was already known to Darwin (1877), who called it the “law of compensation.” CMS has been very well studied in some plant species, because it is a useful tool in the production of hybrid seeds for agriculture (e.g., Hanson 1991). Mitochondrial CMS mutations are often counteracted by so-called nuclear restorer genes (Gigord et al. 1999; Delph and Mutikainen 2003; Koelewijn 2003), which can restore normal SA to different degrees. Such partial CMS is common, but researchers may be more likely to initially detect cases with extreme CMS phenotypes.

In contrast to the widespread occurrence of gynodioecy in plants, there appear to be very few examples among animals. One clear example is the coral Coralliastrum astreoides (Chornesky and Peters 1987), but it is not known whether gynodioecy is due to mtDNA-induced CMS or other factors (such as vertically transmitted mutualists, see below). The sipunculid Golfingia minuta may be another example, although there is some debate about these claims (Hyman 1959): it may instead represent a type of SDSA with young individuals being hermaphrodites and older individuals female (Ghiselin 1969). Finally, gynodioecy may occur among some unionid mussels, but it may be linked to parasitism by trematodes (Kat 1983, see below).

One reason for the scarcity of (evidence for) CMS in simultaneously hermaphroditic animals may simply be the much more advanced knowledge of SA patterns in plants, combined with a
striking and externally visible phenotype (e.g., the lack of stamens and pollen). In animals CMS could be more difficult to detect, as it would generally affect internal organs, such as the testis and/or the male genitalia, and so a CMS phenotype may become apparent only after dissection. However, there is considerable knowledge about the reproductive morphology in at least some groups of simultaneously hermaphroditic animals, such as the gastropods. And in many transparent species, such as small free-living flatworms and chaetognaths, the absence of the testis or male genitalia would also represent a striking phenotype (particularly because the latter are often important for species identification).

In a number of species of gastropods there exists a condition called aphallic, where some individuals in a population do not develop a phallus. However, it is currently unclear if the inheritance pattern of this genital polymorphism is indicative of cytonuclear conflict. Instead there appears to be a strong environmental component to aphally (Ostrowski et al. 2003; Jordaens et al. 2006). Moreover, these studies have not found compensation in the female function in the aphallic individuals, possibly because the investigated species have a small allocation to the male function due to their high selfing rates. High selfing rates lead to strongly female-biased SA, which limits the transmission advantage that a CMS mutation could obtain (because the potential compensation on the female side due to the loss of the male function will be very small).

The presence of conditional sperm trading (Leonard and Lukowiak 1984; Vreys and Michiels 1998; Anthes et al. 2005; but see Anthes and Michiels 2005) could limit the reproductive success of male-sterile individuals, because they cannot reciprocate the sperm donation of their partner, and would thus be rejected as mating partners. In such species one may therefore not expect completely male-sterile individuals (although some degree of CMS may still be possible). It is, however, questionable if conditional sperm trading is widespread enough among internally fertilizing simultaneous hermaphrodites to generally account for the apparent lack of CMS in these organisms. Experimentally preventing the expression of the male function in different species of copulating simultaneous hermaphrodites and measuring their acceptance as mating partners is required to test this.

In the absence of extreme cases of CMS one may have to resort to quantitative genetic studies to partition the phenotypic variation in SA into nuclear, mitochondrial, and environmental components. In the offspring the nDNA will represent a mixture of the parental nDNAs (with some variation due to segregation and recombination), but the mtDNA will come from either one or the other mother, as one can obtain maternal offspring from both partners. Such a crossing design would therefore allow studying the interactions between different mtDNAs within a homogenous nDNA background. Cytonuclear interactions would be expected to lead to different SA patterns depending on the identity of the mother. To the best of my knowledge no such study has been published for a simultaneously hermaphroditic animal.

Finally, the small size of animal mitochondrial genomes (15–17 kb compared to 200–2400 kb in plants), and the degree to which they undergo major gene rearrangements and recombination only in plants (e.g., Ward et al. 1981; Lonsdale et al. 1988; Logan 2006) may explain the scarcity of CMS: there are many more opportunities for mutations in plant mtDNA, compared to the already highly reduced and relatively stable animal mitochondrial genomes.
competition (Charnov 1979; Michiels 1998; Schärer and Janicke 2009). A behavior that has been viewed in this context is the intriguing penis biting in banana slugs of the genus *Ariolimax* (Reise and Hutchinson 2002, J. L. Leonard, pers. comm.). It appears that in some matings the sperm recipient may hold on to the penis of the sperm donor with a strong muscle in the female reproductive tract, and consume it if it is severed. However, this is an exceedingly rare behavior (Reise and Hutchinson 2002, J. L. Leonard, pers. comm.) and its function in manipulation of the partners SA remains speculative. However, this function could be tested by determining if the SA becomes more female biased and the mating rate reduced after experimental removal of the penis.

Michiels (1998) has suggested that recipients may anticipate the donor-induced manipulation by a priori biasing their SA toward the male function. This would predict that virgin individuals (or individuals with a low mating rate) should be more male biased in their SA than frequently mated individuals (which is the opposite prediction from the mating group size models). This prediction is not supported in *M. lignano* (Schärer and Janicke 2009). However, a recent study on the pond snail, *L. stagnalis*, is suggestive of this pattern (Koene et al. 2006): once-mated snails produced a lower total number of eggs than repeatedly grouped snails. However, there are two problems with this interpretation. First, additional research has shown that the number of eggs produced actually drops again at even higher mating rates (J. Koene, pers. comm.), which is more consistent with the expected response to mating group size. Second, this result is also affected by the trade-off between egg number and the investment per egg (see above). So, whereas *L. stagnalis* shows clear evidence that allohormones cause physiological effects in the partner (Koene et al. 2009), it is currently unclear if these effects aim at manipulating SA, or if SA actually changes in response.

And whereas there are at least two other examples among simultaneously hermaphroditic animals of allohormones with effects on the mating partner (Koene et al. 2005; Chase and Blanchard 2006), these allohormones seem to be primarily involved in influencing the patterns of sperm storage, and neither of them has been suggested to have an effect on SA. Given the experimental toolbox that is now available for *L. stagnalis*, this species currently appears most promising to answer these questions.

Michiels (1998) has further suggested that the anticipation of a SA manipulation may explain the widespread occurrence of protandrous simultaneous hermaphroditism, but as outlined above the same pattern is also predicted based on SDSA (Vizoso and Schärer 2007). More experimental tests of SA patterns in virgin versus mated individuals are required to distinguish between these alternative hypotheses. However, particular care has to be taken to use appropriate SA measures (preferably dynamic measures), because in virgins standing stock measures may yield particularly misleading SA estimates.

Because one expects manipulative traits to evolve under sexually antagonistic coevolution there could be genetic variation in male persistence and female resistance traits (Arnqvist and Rowe 2005). One could therefore predict that the SA that an individual exhibits may depend on the genotype of its partner. This could be tested experimentally by a fully factorial crossing design between many different fixed genetic lines.

**PARASITES AND MUTUALISTS**

One largely unexplored factor in hermaphroditic SA is the potential influence of parasites (although a recent example exists in a sequential hermaphrodite, Schärer and Vizoso 2003). Parasitic castration is widespread in many trematode-snail systems (e.g., Sorensen and Minchella 2001) and in cases in which a parasite primarily affects one sex function, simultaneous hermaphrodites may have the ability to escape the parasite by shifting resources to the less affected sex. Although there has been considerable interest in resource allocation in such trematode-snail systems (e.g., Hall et al. 2007), to my knowledge no models have explored the implications for SA. It is conceivable that gastropods are not the most suitable organisms to study this question because they have an ovotestis, and pathogenic effects of the parasites residing in this organ may affect both male and female tissues. However, some parasites appear to specifically influence the expression of neuromodulators, which can directly affect specific reproductive processes and behaviors. A well-understood system is the trematode-induced release of schistosomin, which effectively prevents ovulation and egg laying in the pond snail *L. stagnalis* (Adam 2002). Most studies on this system have so far primarily focused on female aspects of reproduction, but there is evidence that these snails can continue to mate in the male role (J. M. Koene, pers. comm.). Experimental administration of schistosomin may therefore allow to manipulate the SA.

Another intriguing system is parasite-induced hermaphroditism in gonochoristic mussels. In some species among the Unionidae the infection with digenean trematodes can lead to the formation of male gonadal tissue in individuals with an overall female phenotype (and vice versa), and it was speculated that the parasites influence the hormonal sex determination (Kat 1983 and references therein). It would be interesting to examine if trematode infections can also affect the SA in hermaphroditic mussels.

Other promising systems would be cases in which the parasite is known to affect only one sex function, such as some parasitic isopods in the simultaneously hermaphroditic shrimp of the genus *Lysmata* (e.g., Calado et al. 2005, 2006). These parasites lead to...
PERSPECTIVE

different levels of female castration, but parasitized shrimp retain the ability to mate in the male role, and are also accepted as mates by nonparasitized individuals. Parasitized *Lysmata* would thus be expected to shift allocation toward the male function.

In addition to parasites that directly target reproductive organs, many nonsex specific parasites may also affect the male and female reproductive system differentially. In analogy to the time-commitment hypothesis (Day and Aarssen 1997), parasite-induced mortality alone may lead to a shift to male allocation, if the time-commitment necessary to get returns for investment in the male function is less than that needed for the female function. In general, whenever one sex function is affected less by a parasite, one would expect a shift toward that function. Experimental exposure to parasites would allow investigating such parasite-induced shifts in SA, and would thus provide a valuable experimental tool to study SA.

The transmission route of a parasite may also affect SA. Sexually transmitted parasites may benefit from biasing SA toward the function that offers the higher transmission success. For example, a parasite that is primarily transmitted from the sperm donor to the recipient could maybe achieve higher mating rates by biasing allocation toward ejaculate production. Little is known about what factors influence the motivation to mate in the male and female role, and such a parasite may prove a useful tool to experimentally manipulate this.

Similarly, vertically transmitted symbionts (which can be parasites or mutualists) may emphasize the function that increases their transmission success. Vertical transmission occurs mainly via eggs, and such symbionts would therefore be expected to favor a female-biased SA or gynodioecy (analogous to cytoplasmically inherited mtDNA). In gonochorists there is extensive evidence that the cytoplasmic bacterium *Wolbachia* can induce sex-ratio distortion (Hardy 2002). However, to my knowledge *Wolbachia* has so far not been observed in any simultaneously hermaphroditic animals (for a screen in 38 species of gastropods see Schilthuizen 2000). In this context it is very interesting to note that the gynodioecious coral *P. astreoides*, which I mention in the context of CMS, has vertical transmission of its mutualists, the dinoflagellate *Symbiodinium* sp. (Thorndell et al. 2006). CMS may therefore be caused by this mutualist, which surely has a more complex genome compared to the mitochondrial genome of the coral, and which would of course also benefit from shifting the SA of its host toward the female function. Gynodioecy could thus be expected in other coral species with vertical transmission of mutualists, and possibly in other simultaneously hermaphroditic animals that carry mutualists, such as many sponges, cnidarians, acel flatworms, free-living flatworms, marine gastropods, and ascidians (Venn et al. 2000).

**Outlook**

**SEX ALLOCATION AND MATING ROLES**

A largely unexplored aspect of SA in simultaneous hermaphrodites is its potential link to mating role preferences. Each sexually produced offspring has exactly one mother and one father, and therefore half of the fitness in the population must come via the male and half via the female function (Fisher 1930; Dusing 1884). This has recently been termed the Fisher condition (Houston and McNamara 2005); it is a necessary fact in sexual organisms and therefore also applies to simultaneous hermaphrodites. If one combines the Fisher condition with biased SA, it becomes clear that whenever SA deviates from equality, as it evidently does in many of the cases I outlined above, then the overall fitness return per unit investment will be higher for the sex with the lower allocation. This begs the question why not more resources are allocated to that function to equalize the costs. The reason why this does not happen is that the SA optimization argument is based on marginal returns, where one asks from which sex function an organism can obtain the higher return for the next resource unit invested. If some external constraint (such as low density) or some central aspect of the mating system (such as efficient sperm displacement) leads to diminishing returns in the male fitness gain curve, then simply more can be gained from investment to the female than the male function beyond a certain point.

A nice example is the reef fish *Hypoplectrus nigricans*, which is thought to have a strong preference for the male mating role (Leonard 1993). The egg trading mating system of these fish appears to enforce paired mating, producing a complete absence of sperm competition (Petersen 1991). Thus higher investment in the male function does not pay off because it would result in strong LSC. As expected under these conditions, these fish have a strongly female-biased SA, where only about 5–10% of the gonad consists of male tissue and where investment in courtship behavior appears to be equal or biased toward the female role (Fischer 1981, 1987; Petersen 1991). The fitness return per unit investment allocated to the male function must necessarily be 10–20× higher than that for the female function, hence a strong preference for the male role is to be expected.

This scenario is similar to recent arguments about the influence of adult sex ratios for the evolution of sex roles in gonochorists (Kokko and Jennions 2008). Here female-biased adult sex ratios (which can, e.g., result from male-biased mortality) will also lead to higher fitness returns per mated male than per mated female, which will in turn influence the pay-offs that males can obtain from allocating toward paternal care versus obtaining matings (see also Queller 1997). Therefore models of mating preferences in hermaphrodites must take into account the SA and the Fisher condition.
Anthes et al. (2006b) have argued that SA “may [... ] be a weak predictor of immediate [mating] role preferences,” because the mating history, such as being sperm depleted or recently mated, will be a more important factor (my italics). Whereas I consider this a valid point, I nevertheless think that the average [mating] role preferences,” because the central importance of SA as a life-history trait in simultaneously hermaphroditic animals, it is surprising how rarely it has been adequately quantified, even in some otherwise well studied taxa. For example, I am aware of only six species of molluscs where attempts have been made to quantify SA (see Appendix 2). Some additional studies on molluscs have used mating role preferences as a proxy for SA, rather than actually measuring SA (e.g., Angeloni et al. 2003; Ohbayashi-Hodoki et al. 2004; Yusa 2008). Given the above reasoning, it is clear that this can potentially be very misleading (e.g., the preference for the male role in H. nigricans would suggest that it has a male-biased SA, which anatomical SA data strongly contradict). I am convinced that more data on SA patterns would greatly help us to understand long-standing questions regarding mating role preferences in simultaneous hermaphrodites (Charnov 1979; Leonard 1990, 2006; Anthes et al. 2006b).

THE COMPARATIVE APPROACH

Most studies on SA in simultaneously hermaphroditic animals have so far been limited to intraspecific studies. Two notable exceptions are a study on five species in the fish genus Lythrypnus (St. Mary 2000) and one on six species in the fish subfamily Ser- raninae (Petersen 1991). Both studies have identified important correlates of SA evolution. St. Mary (2000) found that species that exist at high density are more polarized into strongly male- and female-biased individuals, whereas species that exist at low density showed more individuals with intermediate SA patterns. These patterns are likely due to strong direct effects of body size linked to higher levels of male–male competition over monopolizations of mating opportunities at high densities, and help to explain the fact that these fish are behaviorally better considered sequential hermaphrodites. And Petersen (1991) showed that the drastically different levels of sperm competition experienced by these usually pair-mating species, which results from different levels of streaking, is a strong predictor of the observed SA patterns. A lot of insight can be gained from comparative analyses about how the mating system and ecological parameters influence SA. Moreover, a molecular phylogenetic approach would further allow identifying how quickly SA can evolve.

SEX ALLOCATION AND THE COST OF SEX

An important conclusion of this review is that SA in simultaneously hermaphroditic animals is frequently expected to deviate from a 50:50 allocation, and that the bias is often toward the female function. This is expected to have consequences for the cost of sexual reproduction in these organisms. In gonochorists, the cost of sex is often assumed to be twofold, because, at least in randomly mating species, Fisherian sex-ratio theory (Dusing 1884; Fisher 1930) predicts that half of the reproductive resources should be invested toward the production of males (which do not directly contribute to population growth unless they provide paternal care). In competition with an asexual lineage, this leads to the twofold cost of sex, which is also often termed the cost of males or the cost of anisogamy (Maynard Smith 1978; Barton and Charlesworth 1998). The situation in simultaneous hermaphrodites is considerably more complex. Although the cost of sex can depend on the SA in a population, it is also expected to depend on the frequency of parthenogenetic hermaphrodites in the population, the fertilization success of the male gametes produced by these individuals (Joshi and Moody 1998), and the population structure (Britton and Mogie 2001).

West et al. (1999) suggested that to explain the maintenance of sex in the light of the cost of sex one needs to consider several hypotheses, some linked to coevolutionary processes with antagonists (e.g., parasites), and others linked to the accumulation of deleterious mutations in asexual lineages. Whereas earlier work has focused on trying to eliminate some of these hypotheses, West et al. (1999) have advocated that empirical work should instead focus on estimating the extent to which these hypotheses can contribute to balance the cost of sex. However, to achieve this aim, one needs to understand how big the cost of sex actually is, and an understanding of the factors that influence SA is essential to answer this question (see also Michiels et al. 1999).

Comparisons of SA patterns in parthenogenetic versus sexual simultaneously hermaphroditic animals are currently restricted to forms with sperm-dependent parthenogenesis (Weinzirl et al. 1998). Regarding the factors that may influence SA, I am aware of only one study that has investigated sperm competitiveness in sexual versus parthenogenetic forms of a simultaneously hermaphroditic animal (D’Souza et al. 2008), comparing two parthenogenetic populations of a sperm-dependent parthenogen, the planarian Schmidtea polychroa, one of which exhibits occasional sex (D’Souza et al. 2004), whereas the other is purely asexual. The study found higher sperm quality in the occasionally sexual population, potentially suggesting that the male function remains an important fitness component in such populations. More data of this type comparing sexuals with more “normal” parthenogens (i.e., not sperm dependent) would be highly interesting.

Conclusions

SA theory clearly offers a powerful framework to understand variation in SA in simultaneously hermaphroditic animals, and
a number of central predictions, such as the link between small mating group size and female-biased SA or that SA varies with body size are, at least qualitatively, well supported. Other predictions, such as the link between selfing rate and SA or the effect of brooding have obtained very little attention. Moreover, not enough effort has been spent on testing central assumptions of SA theory such as the presence of the SA trade-off, or the suitability of the chosen allocation measures. More quantitative tests of SA theory will only be possible with a better understanding of the parameters that are measured and a wider range of approaches (e.g., quantitative genetics, artificial selection, direct manipulation of allocation). Finally, a widening of the taxonomic scope of SA research would be highly welcome to more clearly document the extent of variation in SA among simultaneously hermaphroditic animals.

ACKNOWLEDGMENTS
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LITERATURE CITED
———. 1877. The different forms of flowers on plants of the same species. John Murray, Lond.


Appendix 1: Glossary.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptic female choice</td>
<td>Nonrandom paternity biases resulting from female morphology, physiology, or behavior that occur after coupling</td>
</tr>
<tr>
<td>Dioecy</td>
<td>Having separate sexes; individuals are either males or females; used for plants. See also gonochorism</td>
</tr>
<tr>
<td>Gonochorism</td>
<td>Having separate sexes; individuals are either males or females; used for animals. See also dioecy</td>
</tr>
<tr>
<td>Gonochorist</td>
<td>An organism whose reproductive mode is gonochorism</td>
</tr>
<tr>
<td>Gynodoecy</td>
<td>Having both hermaphroditic individuals and females in the same species; rare in animals</td>
</tr>
<tr>
<td>Local mate competition (LMC)</td>
<td>Competition between related individuals for access to mates</td>
</tr>
<tr>
<td>Local resource competition (LRC)</td>
<td>Related individuals (that derive) of one sex compete more for resources</td>
</tr>
<tr>
<td>Local sperm competition (LSC)</td>
<td>Competition between related sperm for the fertilization of a given set of ova</td>
</tr>
<tr>
<td>Mating group size</td>
<td>The number of sperm donors per sperm recipient plus 1</td>
</tr>
<tr>
<td>Protandrous simultaneous Hermaphrodite</td>
<td>An organism that is male early in life, and a simultaneous hermaphrodite later in life</td>
</tr>
<tr>
<td>Sequential hermaphrodite</td>
<td>An organism in which individuals exhibit one sex early in life, and change sex later in life</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>The ratio of the sexes in a group of organisms (often applied to the offspring of one mother)</td>
</tr>
<tr>
<td>Simultaneous hermaphrodite</td>
<td>An organism in which individuals exhibit both sexes during at least part of their life; as most animals are not modular, there is generally no distinction between hermaphroditism and monoecy, as is often used in plants</td>
</tr>
<tr>
<td>Social group size</td>
<td>The number of individuals that an individual interacts but not necessarily mates with</td>
</tr>
<tr>
<td>Sperm competition</td>
<td>Competition between the sperm of two or more (unrelated) individuals for the fertilization of a given set of ova</td>
</tr>
<tr>
<td>Sperm displacement</td>
<td>The process of a volumetric exchange of previously stored sperm by new sperm</td>
</tr>
<tr>
<td>Spermcast mating</td>
<td>A plant-like mating system in marine invertebrates with release of sperm into the water, with subsequent sperm uptake, and internal fertilization</td>
</tr>
</tbody>
</table>

Associate Editor: M. Rausher
## Appendix 2: Studies on sex allocation (SA) in simultaneously hermaphroditic animals

Grouped by the aspect of sex allocation theory that they test and sorted by the hypothesis being tested, the study type (c, comparative; d, descriptive; e, experimental); and the publication year. Mating system (ms): o, outcrossing; po, primarily outcrossing; fs, facultatively selfing; ps, primarily selfing; sp, sperm-dependent parthenogenesis. Studies that test multiple hypothesis may appear repeatedly. The references cited in this appendix are listed in the (References S1).

<table>
<thead>
<tr>
<th>Hypothesis Type</th>
<th>Taxon</th>
<th>ms</th>
<th>SA measure(s)</th>
<th>Result</th>
<th>Support</th>
<th>Comment/critique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Studies testing assumptions about SA measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed costs d</td>
<td>Freshwater snail, <em>Bulinus truncatus</em></td>
<td>ps</td>
<td>Weight of ♀ genital tract</td>
<td>♀ allocation not higher in aphallic snails</td>
<td>No</td>
<td>Cost of ♂ function may be low due to selfing, gametic SA not measured</td>
<td>Doums and Jamne 1996</td>
</tr>
<tr>
<td>Testis size reflects ♂ allocation e</td>
<td>Free-living flatworm, <em>Macrostomum sp.</em></td>
<td>o</td>
<td>Testicular activity</td>
<td>Bigger testis more active</td>
<td>Yes</td>
<td>Dynamic measure</td>
<td>Schärer et al. 2004</td>
</tr>
<tr>
<td>Testis size reflects ♂ allocation e</td>
<td>Free-living flatworm, <em>Macrostomum sp.</em></td>
<td>o</td>
<td>Testis and ovary area</td>
<td>Trade-off present</td>
<td>Yes</td>
<td>Only visible under specific conditions</td>
<td>Schärer et al. 2005</td>
</tr>
<tr>
<td>B: Studies testing assumptions about the SA trade-off</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA trade-off e</td>
<td>Freshwater snail, <em>Lymnea stagnalis</em></td>
<td>fs</td>
<td>Egg production</td>
<td>Castration doubles egg number</td>
<td>Yes?</td>
<td>Experimentally prevents mating behavior, male function not measured</td>
<td>De Visser et al. 1994</td>
</tr>
</tbody>
</table>

Continued.
### Appendix 2: Continued.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Type</th>
<th>Taxon</th>
<th>ms</th>
<th>SA measure(s)</th>
<th>Result</th>
<th>Support</th>
<th>Comment/critique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C: Studies testing predictions related to a saturating male fitness gain function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monogamy</td>
<td>d</td>
<td>Fish, <em>Hypoleucrus nigricans</em></td>
<td>o</td>
<td>Gonad morphology, courtship behavior</td>
<td>♀-biased SA</td>
<td>Yes</td>
<td>Testis 10% of gonad, courtship equal</td>
<td>Fischer 1981</td>
</tr>
<tr>
<td>&quot;</td>
<td>d</td>
<td>Polychaete, <em>Ophryotrocha puerelis puerelis</em></td>
<td>o</td>
<td>Effect of ♂ and ♀ allocation on growth</td>
<td>♀ function more costly</td>
<td>Yes</td>
<td>Sequential hermaphrodite turns into simultaneous hermaphrodite</td>
<td>Berglund 1986</td>
</tr>
<tr>
<td>&quot;</td>
<td>d</td>
<td>Polychaete, <em>Ophryotrocha diadema</em></td>
<td>o</td>
<td>Histology of sperm and ovarian tissue</td>
<td>♀-biased SA</td>
<td>Yes</td>
<td></td>
<td>Sella 1990</td>
</tr>
<tr>
<td>Parthenogenesis</td>
<td>d</td>
<td>Free-living flatworm, <em>Schmidtea polychroa</em></td>
<td>o/sp</td>
<td>Testis and vas deferens size</td>
<td>♂ allocation low in parthenogens</td>
<td>Yes</td>
<td>Only three populations</td>
<td>Weinzierl et al. 1998</td>
</tr>
<tr>
<td>&quot;</td>
<td>d</td>
<td>Free-living flatworm, <em>Schmidtea polychroa</em></td>
<td>sp</td>
<td>Cocoon production</td>
<td>♀ allocation high in parthenogens</td>
<td>Yes</td>
<td>Only one population</td>
<td>Weinzierl et al. 1998</td>
</tr>
<tr>
<td>Selfing rate</td>
<td>d</td>
<td>Bivalve, <em>Utterbackia imbecillis</em></td>
<td>fs</td>
<td>Testicular and ovarian tissue</td>
<td>Selfers have more ♀-biased SA</td>
<td>Yes</td>
<td>Small sample size, n = 4</td>
<td>Johnston et al. 1998</td>
</tr>
<tr>
<td>Spawning</td>
<td>c</td>
<td>Reef fish, Serraninae</td>
<td>o</td>
<td>Testis and ovary dry weight</td>
<td>More ♂ with high sperm competition</td>
<td>Yes</td>
<td>Phylogeny not controlled</td>
<td>Petersen 1991</td>
</tr>
<tr>
<td>Sperm</td>
<td>d</td>
<td>Reef fish, <em>Serranus tortugarum</em></td>
<td>o</td>
<td>Theoretical prediction</td>
<td>Hermaphroditism stable</td>
<td>Yes</td>
<td></td>
<td>Fischer 1984</td>
</tr>
<tr>
<td>competition</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>e</td>
<td>Bryozoa, <em>Celleporella hyalina</em></td>
<td>o</td>
<td>Number of ♂ and ♀ zooids</td>
<td>♂ allocation up</td>
<td>Yes</td>
<td>One stressor was presence of conspecific</td>
<td>Hughes et al. 2003</td>
</tr>
<tr>
<td>Mating rate</td>
<td>e</td>
<td>Land snail, <em>Arianta arbustorum</em></td>
<td>po</td>
<td>Spermatophore size, egg production</td>
<td>SA not affected</td>
<td>No</td>
<td>♀-biased SA</td>
<td>Locher and Baur 2000a</td>
</tr>
<tr>
<td>&quot;</td>
<td>e</td>
<td>Sea slug, <em>Aeolidiella glauca</em></td>
<td>o</td>
<td>Spermatophore weight, egg number</td>
<td>♂ allocation higher ♀ allocation lower</td>
<td>Unclear</td>
<td>Only in one year, mortality affected by treatment</td>
<td>Karlsson 2001</td>
</tr>
<tr>
<td>&quot;</td>
<td>e</td>
<td>Freshwater snail, <em>Lymnea stagnalis</em></td>
<td>fs</td>
<td>Prostate gland weight, egg production, area of ♂ and ♀ ovotestis components</td>
<td>Prostate gland weight down, egg production up, ovotestis unaffected</td>
<td>No</td>
<td>Histology requires systematic sampling</td>
<td>Koene et al. 2006</td>
</tr>
<tr>
<td>Mating group size</td>
<td>d</td>
<td>Barnacle, <em>Catomerus polymerus</em></td>
<td>?</td>
<td>Dry weight of eggs, sperm, testis and seminal vesicle</td>
<td>♂ allocation up ♀ allocation down</td>
<td>Yes</td>
<td>Confounded with density</td>
<td>Raimondi and Martin 1991</td>
</tr>
</tbody>
</table>
### Appendix 2: Continued.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Type</th>
<th>Taxon</th>
<th>ms</th>
<th>SA measure(s)</th>
<th>Result</th>
<th>Support</th>
<th>Comment/critique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Tremadode, <em>Echinostoma caproni</em></td>
<td>fs</td>
<td>Ovary, testis, and cirrus sack area, fecundity</td>
<td>♂ allocation up, ♀ allocation down</td>
<td>Yes</td>
<td>Confounded with size and density</td>
<td>Trouvé et al. 1999</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Land snail, <em>Arianta arbustorum</em></td>
<td>po</td>
<td>Spermatophore size, egg production</td>
<td>♂ allocation same, ♀ allocation up</td>
<td>No</td>
<td>Mucus trails as cue for mating group size Social facilitation?</td>
<td>Locher and Baur 2000b</td>
</tr>
<tr>
<td>Mating group size</td>
<td>e</td>
<td>Cestode, <em>Schistocephalus solidus</em></td>
<td>fs</td>
<td>Testis, yolk gland, and ovary volume</td>
<td>SA not affected</td>
<td>No</td>
<td>Small sample size</td>
<td>Schärer and Wedekind 2001</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Free-living flatworm, <em>Macrostomum sp.</em></td>
<td>o</td>
<td>Testis and ovary area</td>
<td>♂ allocation up, ♀ allocation down</td>
<td>Yes</td>
<td>No effect on the ♀ function</td>
<td>Schärer and Ladurner 2003</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Leech, <em>Helobdella papillornata</em></td>
<td>fs</td>
<td>Testis sac volume, total egg volume</td>
<td>♂ allocation up, ♀ allocation same</td>
<td>Yes</td>
<td>Confounded with density</td>
<td>Tan et al. 2004</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Free-living flatworm, <em>Macrostomum sp.</em></td>
<td>o</td>
<td>Testicular activity</td>
<td>Bigger testis more costly</td>
<td>Yes</td>
<td>Dynamic measure</td>
<td>Schärer et al. 2004</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Freshwater snail, <em>Lymnea stagnalis</em></td>
<td>fs</td>
<td>Prostate and albumen gland weight</td>
<td>Prostate gland weight down, albumen gland weight unclear</td>
<td>No</td>
<td>Pseudoreplicated, posthoc split in analysis, effect on body size</td>
<td>Koene and Ter Maat 2004</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Polychaete, <em>Ophryotrocha diadema</em></td>
<td>o</td>
<td>Sperm and cocoon number</td>
<td>♂ allocation same, ♀ allocation down</td>
<td>No?</td>
<td>Drastic drop in ♀ SA despite no effect in ♂ SA, combined SA measure therefore flawed</td>
<td>Lorenzi et al. 2005</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Polychaete, <em>Ophryotrocha diadema</em></td>
<td>o</td>
<td>Cocoon and egg number</td>
<td>♀ allocation down</td>
<td>Yes</td>
<td>♂ SA not quantified, species-specific chemical signal</td>
<td>Schleicherova et al. 2006</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>e</td>
<td>Polychaete, <em>Ophryotrocha diadema</em></td>
<td>o</td>
<td>Sperm and cocoon number, behavior</td>
<td>♂ allocation not higher, ♀ allocation down, more aggression</td>
<td>Yes</td>
<td>Suggests that ♂ allocation may go toward aggression rather than sperm</td>
<td>Lorenzi et al. 2006</td>
</tr>
</tbody>
</table>

Continued.
### Appendix 2: Continued.

<table>
<thead>
<tr>
<th>Hypothesis</th>
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<th>Result</th>
<th>Support</th>
<th>Comment/critique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;e&quot;</td>
<td>Free-living flatworm, <em>Macrostomum lignano</em></td>
<td>o</td>
<td>Sperm production rate</td>
<td>Bigger testis produce more sperm</td>
<td>Yes</td>
<td>Testis size is a good but incomplete measure of sperm production rate</td>
<td>Schärer and Vizoso 2007</td>
<td></td>
</tr>
<tr>
<td>&quot;e&quot;</td>
<td>Shrimp, <em>Lysmata wurdemanni</em></td>
<td>o</td>
<td>Sperm and egg dry weight</td>
<td>SA not affected</td>
<td>No</td>
<td>Sperm competition absent also in large groups</td>
<td>Baeza 2007b</td>
<td></td>
</tr>
<tr>
<td>&quot;e&quot;</td>
<td>Free-living flatworm, <em>Macrostomum lignano</em></td>
<td>o</td>
<td>Testis and ovary area</td>
<td>Flexible SA adjustment</td>
<td>Yes</td>
<td>Flexibility may be somewhat constrained</td>
<td>Brauer et al. 2007</td>
<td></td>
</tr>
<tr>
<td>&quot;e&quot;</td>
<td>Polychaete, <em>Ophryotrocha diadema</em></td>
<td>o</td>
<td>Fertilization success, egg number</td>
<td>Flexible SA adjustment</td>
<td>Yes?</td>
<td>Analysis flawed, lack of constraint not shown</td>
<td>Lorenzi et al. 2008</td>
<td></td>
</tr>
<tr>
<td>Fitness gain curve</td>
<td>d/e</td>
<td>Bryozoan, <em>Celleporella hyalina</em></td>
<td>po</td>
<td>Number of ♀ zooids</td>
<td>Reduced gain under competition</td>
<td>Yes</td>
<td>Focal zooid number not manipulated</td>
<td>Yund and McCartney 1994</td>
</tr>
<tr>
<td>&quot;d&quot;</td>
<td>Bryozoan, <em>Celleporella hyalina</em></td>
<td>po</td>
<td>Number of ♀ zooids</td>
<td>No nonlinearity</td>
<td>No</td>
<td>Zoid number not manipulated</td>
<td>McCartney 1997</td>
<td></td>
</tr>
<tr>
<td>&quot;e&quot;</td>
<td>Ascidian, <em>Botryllus schlosseri</em></td>
<td>o</td>
<td>Testis area</td>
<td>Competition leads to linearization</td>
<td>Yes</td>
<td>Focal testis size not manipulated</td>
<td>Yund 1998</td>
<td></td>
</tr>
</tbody>
</table>

**D: Studies testing predictions related to a saturating female fitness gain function**

| "d" | Coral, *Stylophora pistillata* | o? | Egg and testis volume | ♀-biased SA | Yes/no | No explicit test, one nonbrooding species also has ♀-biased SA | Hall and Hughes 1996 |
| "d" | Shrimp, *Lysmata wurdemanni* | o | ♀ fecundity | ♀-biased SA | No | Brood capacity only weakly constrained | Baeza 2007c |

**E: Studies testing for the effects of size and budget**

| Mating in the ♀ role costly | d | Polychaete, *Ophryotrocha diadema* | o | Growth | Growth delayed when mated | Yes? | ♀ allocation not measured, no statistical test for comparison of experimental vs. control | Sella and Lorenzi 2003 |
| "d" | Polychaete, *Ophryotrocha diadema* | o | Fertilization success, cocoon number | Protandrous mating reduces life span and ♀ reproductive success | Yes | Actual ♀ allocation not measured, but assumed to be proportional to fertilization success | Lorenzi et al. 2006 |

| Population density | c | Fish, Gobiidae | o | Testis area | SA more extreme at higher density | Yes | | St. Mary 2000 |

*Continued.*
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<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Resource level</td>
<td>e Bryozoa, Celleporella hyalina</td>
<td>po</td>
<td>Zooid sex-ratio</td>
<td>SA not affected</td>
<td>No</td>
<td>SA affected by age and genotype</td>
<td>Hughes and Hughes 1986</td>
</tr>
<tr>
<td>”</td>
<td>e Bryozoa, Celleporella hyalina</td>
<td>po</td>
<td>Zooid sex-ratio</td>
<td>SA not affected</td>
<td>No</td>
<td>SA affected by genotype</td>
<td>Cancino and Hughes 1987</td>
</tr>
<tr>
<td>”</td>
<td>e Bryozoa, Celleporella hyalina</td>
<td>po</td>
<td>Zooid sex-ratio</td>
<td>SA not affected</td>
<td>No</td>
<td>SA affected by genotype</td>
<td>Hughes 1989</td>
</tr>
<tr>
<td>”</td>
<td>e Bryozoa, Celleporella hyalina</td>
<td>po</td>
<td>Number of ♀ and ♂ zooids</td>
<td>Confusing results</td>
<td>Yes?</td>
<td>Some evidence for SA trade-off</td>
<td>Hunter and Hughes 1995</td>
</tr>
<tr>
<td>”</td>
<td>e Land snail, Arianta arbustorum</td>
<td>po</td>
<td>Spermatophore and egg mass and N content</td>
<td>♀ allocation same ♀ allocation down</td>
<td>Yes?</td>
<td>Only ♀ function affected</td>
<td>Locher and Baur 2002</td>
</tr>
<tr>
<td>”</td>
<td>e Colonial ascidian, Botryllus schlosseri</td>
<td>o</td>
<td>Testis area, egg production</td>
<td>♀ allocation down ♀ allocation up</td>
<td>Yes</td>
<td>No trade-off, actual resource level not measured</td>
<td>Newlon et al. 2003</td>
</tr>
<tr>
<td>”</td>
<td>e Free-living flatworm, Macrostomum lignano</td>
<td>o</td>
<td>Testis and ovary area</td>
<td>Protandry, more ♀-biased SA with higher resources</td>
<td>Yes</td>
<td>Evidence for SDSA</td>
<td>Vizoso and Schärer 2007</td>
</tr>
<tr>
<td>SDSA</td>
<td>d Reef fish, Serranus fasciatus</td>
<td>o</td>
<td>Testis and ovary dry weight</td>
<td>Testis 5–100% of gonad</td>
<td>Yes</td>
<td>Hermaphrodites and males</td>
<td>Petersen 1990</td>
</tr>
<tr>
<td>SDSA</td>
<td>d Coral, Acropora hyacinthus</td>
<td>o</td>
<td>Egg and testis volume</td>
<td>Larger more ♀-biased</td>
<td>Yes</td>
<td>Similar trend for other species</td>
<td>Hall and Hughes 1996</td>
</tr>
<tr>
<td>”</td>
<td>d/e? Shrimp, Lysmata wurdemanni</td>
<td>o</td>
<td>Resource holding power of ♂ vs. hermaphroditic phase</td>
<td>Hermaphrodites better</td>
<td>Yes?</td>
<td>Methods state comparison between small and large hermaphrodites, phase (or size) not manipulated</td>
<td>Baeza 2006</td>
</tr>
<tr>
<td>”</td>
<td>d/e? Shrimp, Lysmata wurdemanni</td>
<td>o</td>
<td>Growth rate of ♂ vs. hermaphroditic phase</td>
<td>Males grow faster</td>
<td>Yes</td>
<td>Phase not manipulated</td>
<td>Baeza 2006</td>
</tr>
<tr>
<td>”</td>
<td>d/e? Shrimp, Lysmata wurdemanni</td>
<td>o</td>
<td>Mortality of ♂ vs. hermaphroditic phase</td>
<td>No difference</td>
<td>No</td>
<td>Phase not manipulated</td>
<td>Baeza 2006</td>
</tr>
<tr>
<td>”</td>
<td>d/e? Shrimp, Lysmata wurdemanni</td>
<td>o</td>
<td>Mortality of small vs. large hermaphroditic phase</td>
<td>Small have higher mortality</td>
<td>Yes</td>
<td>Size not manipulated</td>
<td>Baeza 2006</td>
</tr>
</tbody>
</table>
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</tr>
</thead>
<tbody>
<tr>
<td>&quot;d/e?</td>
<td></td>
<td>Shrimp, <em>Lysmata wurdemanni</em></td>
<td>o</td>
<td>Time-commitment of $\sigma$ vs. $\varphi$ function</td>
<td>$\varphi$ function takes longer</td>
<td>Yes</td>
<td>Phase not manipulated</td>
<td>Baeza 2006</td>
</tr>
<tr>
<td>&quot;e</td>
<td></td>
<td>Fish, <em>Lythrypnus dalli</em></td>
<td>o</td>
<td>Testis area</td>
<td>Reallocate according to relative size</td>
<td>Yes</td>
<td>Body size not manipulated</td>
<td>St. Mary 1994</td>
</tr>
<tr>
<td>SDSA / local mate competition</td>
<td>d</td>
<td>Cestode, <em>Schistocephalus solidus</em></td>
<td>fs</td>
<td>Testis, yolk gland, and ovary volume</td>
<td>$\varphi$-biased SA and larger more $\varphi$-biased</td>
<td>Yes</td>
<td></td>
<td>Schärer et al. 2001</td>
</tr>
<tr>
<td>SDSA / mating opportunity</td>
<td>e</td>
<td>Shrimp, <em>Lysmata wurdemanni</em></td>
<td>o</td>
<td>Timing of $\varphi$ maturation</td>
<td>$\varphi$ maturation delayed</td>
<td>Yes</td>
<td>Also differences in growth rate</td>
<td>Baeza 2007a</td>
</tr>
<tr>
<td>SDSA / monogamy</td>
<td>d</td>
<td>Shrimp, <em>Lysmata wurdemanni</em></td>
<td>o</td>
<td>Sperm, testis, and ovary dry weight</td>
<td>$\varphi$-biased SA, larger more $\varphi$-biased</td>
<td>Yes</td>
<td>Size not manipulated</td>
<td>Baeza 2007c</td>
</tr>
<tr>
<td>SDSA / sperm competition</td>
<td>c</td>
<td>Reef fish, <em>Serranus tortugarum</em></td>
<td>o</td>
<td>Testis and ovary dry weight</td>
<td>Larger more $\varphi$-biased</td>
<td>Yes</td>
<td></td>
<td>Petersen and Fischer 1996</td>
</tr>
<tr>
<td>&quot;d</td>
<td></td>
<td>Reef fish, <em>Serranus subligarus</em></td>
<td>o</td>
<td>Testis and ovary dry weight</td>
<td>Testis 8% of gonad</td>
<td>Yes</td>
<td>No effect of body size</td>
<td>Oliver 1997</td>
</tr>
</tbody>
</table>

**F:** Studies testing SA responses not currently predicted by SA theory

| Inbreeding | e | Bryozoan, *Celleporella hyalina* | o  | Number of $\sigma$ and $\varphi$ zooids | More $\sigma$ when inbred | Yes | May be due to stress | Hoare and Hughes 2001 |
| Sperm receipt from nonrelative | e | Bryozoan, *Celleporella hyalina* | o  | Number of $\sigma$ and $\varphi$ zooids | More $\varphi$ zooids | Yes | | Hughes et al. 2002 |
| Various stressors | e | Bryozoan, *Celleporella hyalina* | o  | Number of $\sigma$ and $\varphi$ zooids | $\sigma$ allocation up | Yes | One stressor was presence of conspecific | Hughes et al. 2003 |
| Flexible ‘fixed’ costs | d | Barnacle, *Semibalanus balanoides* | o  | Penis length | Shorter at high population densities | Yes | Mating group size might predict the opposite | Hoch 2008 |

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1 *Macrostomum* sp. has in the meantime been named *Macrostomum lignano*, and *Anodonta imbecilis* is synonymous with *Utterbackia imbecillis*.

2 A renanalysis using a nested model and thus avoiding pseudoreplication confirms the conclusions (J. M. Koene, pers. comm).
Supporting Information
The following supporting information is available for this article:

References S1. The complete list of references cited in Appendix 2.
Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).
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Additional results and discussion can be found in a document at http://www.repository.naturalis.nl/record/289893.