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The relationship between sperm reservoir and spermatophore length in benthic octopuses (Cephalopoda: Octopodidae)

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The relationship between sperm reservoir and total spermatophore length among 168 spermatophores from 44 species in 11 genera has been considered. Bivariate plots show that four Atlantic species of the genus *Eledone* produce spermatophores with relatively large sperm reservoirs that differ from all others. Measurements of the remaining spermatophores are so tightly correlated that a single equation explains over 96% of the variation. Functional constraints on gross spermatophore morphology may be so strong that males cannot manipulate sperm reservoir size independently of spermatophore size to maximize the sperm delivered at a single copulation. Alternate means to assure male paternity may have evolved in the group as a result. Despite the overall uniformity of the relationship among all species other than those of *Eledone* in the Atlantic, these measurements distinguish the overtly similar species *Octopus bimaculatus*–*Octopus bimaculoides* and separate *Benthoctopus januarii* from all others.

INTRODUCTION

Octopodid spermatophores are composed of a thick, opaque, white sperm reservoir, which holds a coiled sperm cord, a middle-piece (Pickford, 1945) and a thin, transparent ejaculatory apparatus. Several measurements have been defined to quantify spermatophore morphology, but presently only total spermatophore length, sperm reservoir length and, more rarely, the maximum width of the spermatophore are routinely reported. This paper explores the relationship between total spermatophore length and sperm reservoir length in 168 spermatophores of 44 species in 11 octopodid genera. The data, a combination of original measurements and literature reports, must be robust to investigator bias to display intelligible pattern. The simple relationship discovered reveals an unsuspected constraint in octopodid reproduction and illustrates the need not only for critical examination of characters in standard use but for the identification of additional, more informative characters.

MATERIALS AND METHODS

The total spermatophore and sperm reservoir lengths for 117 specimens were compiled from literature reports and an additional 51 spermatophores were measured. Appendix 1 lists the 44 octopodid species in 11 genera taxa considered and the data source(s) for each. The genera included and their numeric representation are *Octopus* (N=107); *Eledone* (N=23); *Benthoctopus* (N=16); *Graneledone* (N=9); *Pareledone* (N=3); *Ameloctopus* (N=2); *Bathypolypus* (N=2); *Enteroctopus* (N=2); *Vosseledone* (N=2); *Vulcanoctopus* (N=1); and *Cistopus* (N=1). Although Pickford (1945) stated that the endpoints of some spermatophore measurements she cited were arbitrary, the sperm reservoir constitutes a well-defined section of the spermatophore and therefore was considered a priori to be homologous among specimens of different taxa and to have been measured in the same way by different authors.

The data were entered into MicroSoft Excel (copyright 1997) and transformed to logarithms of base 10. Log transformation equalizes error over the wide range of sizes considered (from 1.5 to 150 mm) while maintaining allometric relationships. The spermatophore and sperm reservoir lengths reported for *Enteroctopus dofleini* (Wülker, 1910) by Sasaki (1929) are roughly seven and a half times larger than any other in the data set, i.e. the spermatophore length for *E. dofleini* is 1130 mm; the next longest are the 150 mm spermatophores of *Benthoctopus macrophallus* Voss & Pearcy, 1990. Although log-transformation tends to minimize the effect of outliers, to ensure that relationships were estimated in the most conservative manner, data from *E. dofleini* were excluded. Sperm reservoir length was then plotted vs total spermatophore length for the remaining species to reveal trends in the relationship between the two variables.

RESULTS

Two relationships between sperm reservoir and total spermatophore length are evident (Figure 1). Spermatophores from four species of the genus *Eledone* from the Atlantic Ocean are unique among those considered in having relatively long sperm reservoirs; over 98.8% of the variation in sperm reservoir length is explained by associated variation in spermatophore length. The other 159 spermatophores representing 40 species, including *Eledone palari* Lu & Stranks, 1991 described from 100 to 600 m depth off Australia and reported from 200 to 300 m depth from the Arafura Sea between 8 and 10°S

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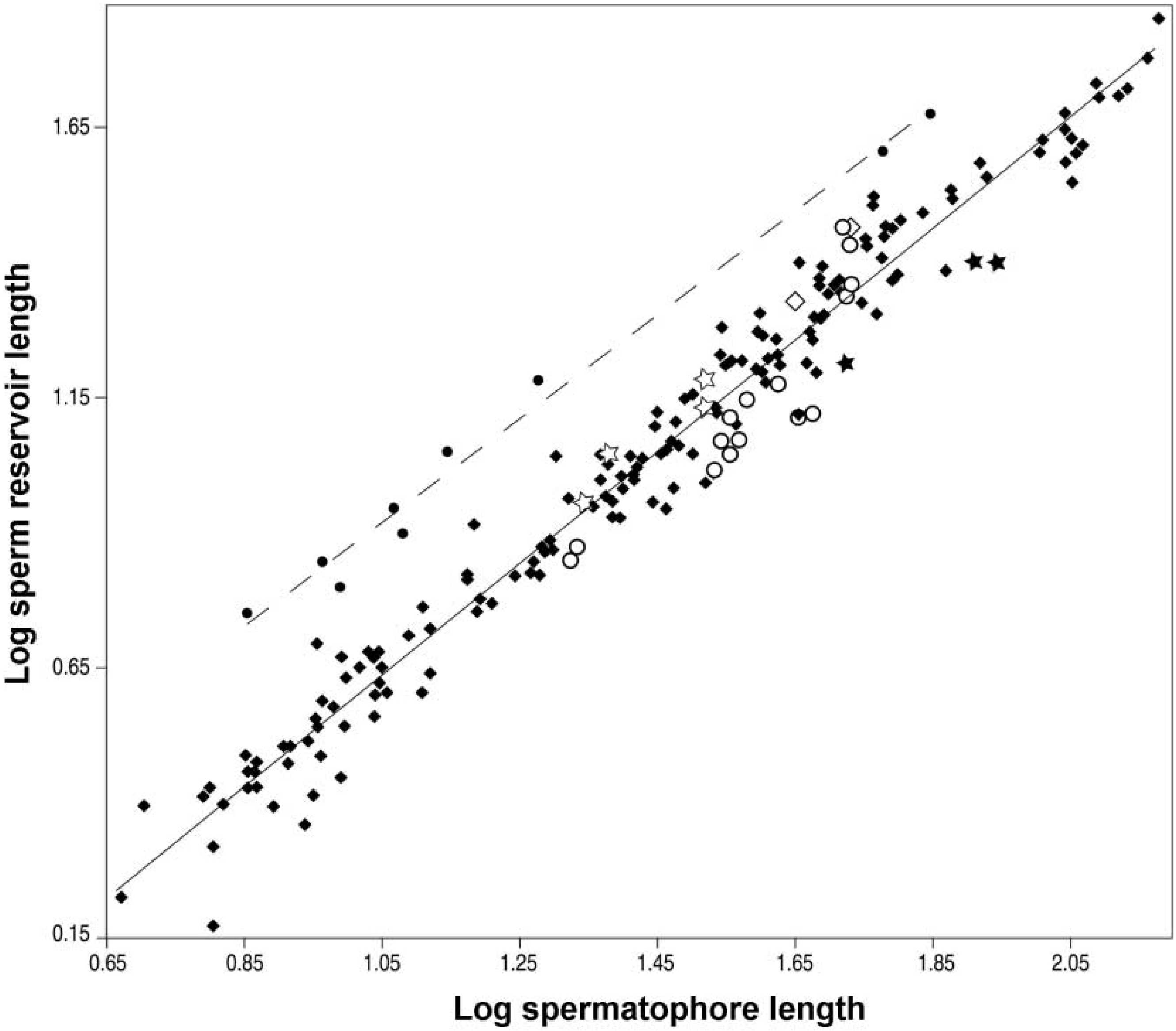

Figure 1. Log sperm reservoir length is plotted vs log spermatophore length. The dashed line indicates the relationship among the data representing four Atlantic species of *Eledone* (closed circles; N=9). The solid line indicates the relationship among the data for all other species considered (solid diamonds; N=134), except *Enteroctopus dofleini*. Taxa that the available data indicate to be outstanding are: *Octopus bimaculoides* Pickford & McConnaughey, 1949 (open stars; N=4); *O. bimaculatus* Verrill, 1883 (open circles; N=16); *Benthoctopus januarii* (Hoyle, 1885) (solid stars; N=3); and *Bathypolypus arcticus* (Prosch, 1849) (open diamonds; N=2). The equation that describes the relationship among the latter group of spermatophores is reported in the text.

(Norman et al., 1997), show a single very strong relationship between spermatophore and sperm reservoir length. The mathematical relationship between these log-transformed variables (excluding data from *Enteroctopus dofleini* and the Atlantic species of *Eledone*) is:

$$SRL = 1.0254\ SPL - 0.4384 \quad (1)$$

where SRL = sperm reservoir length; SPL = spermatophore length, the sum of sperm reservoir length, ejaculatory apparatus length and middle-piece length.

This equation accounts for 96.45% of the variation in the plot (Figure 1). Apparently sperm reservoir and spermatophore lengths are tightly coupled in all of these octopodid taxa.

Among the raw data, exclusive of *Enteroctopus dofleini* and the Atlantic species of *Eledone*, 94.55% of the variation in sperm reservoir length is accounted for by total spermatophore length. Including the two very large data points representing *E. dofleini* artificially increases the variation explained to 98.8% in the non-log-transformed data. In contrast, adding these data to the log-transformed data set increases the variation explained by only 0.69%. The diverse sources of the measurements considered here and the difficulties inherent in measuring these thin, curved structures make the strength and consistency of the relationship all the more surprising.

Despite the very strong relationship documented, the sibling species *Octopus bimaculatus* Verrill, 1883 and *O. bimaculoides* Pickford & McConnaughey, 1949 can be distinguished (Figure 1) by the longer sperm reservoir in the shorter spermatophore produced by the latter. In addition, all three spermatophores of *Benthoctopus januarii* (Hoyle, 1885) reported by Toll (1981) are smaller than

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expected for the size of the spermatophore; those of the two individuals of the genus *Bathypolypus* considered are larger than expected. Spermatophores shorter than 85 mm produced by species of the *Octopus macropus* Risso, 1826 group have very slightly larger sperm reservoirs than expected, and spermatophores over 38 mm long produced by some species of the *Octopus vulgaris* Cuvier, 1797 group may have very slightly smaller reservoirs than expected. Although individual spermatophores of other taxa may occasionally be unusual, the taxa noted are the only ones which appear to show consistent differences.

Despite these subtle apparent differences among species, the uniformity of the isometric relationship between sperm reservoir and spermatophore length throughout the family suggests that spermatophore morphology, and the process of spermatophore formation, are strongly conserved. Because spermatophores release sperm due to osmotic pressures created by concentrated, high molecular weight fluid within the semipermeable membrane of the spermatophore tunic (Mann, 1984), the concentration and volume of fluid relative to the volume of sperm in a spermatophore may be strongly canalized. Because spermatophores of the Atlantic species of *Eledone* are seemingly unique among octopods in that they deliver sperm to ovarian eggs rather than to spermathecae in the oviduct (Perez et al., 1990), their morphology is considered to be derived. Only a very short portion of the small spermatophores in these species is occupied by what has been described as a nearly unformed ejaculatory apparatus, yet their function is unimpaired (Fort, 1937, 1941; Mangold-Wirz, 1963). A study comparable to that of Mann (1984) documenting the osmolality of the spermatophore fluid in these taxa may be the best means to address this question. Taxonomic accounts have reported the sperm reservoir to occupy a larger proportion of the spermatophore in *Eledone cirrhosa* (Lamarck, 1798) than in *E. moschata* (Lamarck, 1799). The contrast between the 60–70 mm long spermatophores of the former species and the 15–20 mm long spermatophores of the latter (Fort, 1937, 1941) appears to offer a better means to distinguish these species.

Because sperm reservoir length appears to be nearly fixed relative to spermatophore length, increasing spermatophore length would seem to be the simplest means for a male to deliver more sperm to a female during copulation. Mangold-Wirz (1963), however, demonstrated that in several species of octopus, spermatophore length reaches a maximum regardless of male size. If spermatophore length is fixed, individual males are likely to be unable to deliver more sperm to females by increasing spermatophore size.

To maximize both the potential taxonomic benefit and insight into reproductive biology that are gained from studies of spermatophores, additional features need to be treated in detail. The details of the ejaculatory apparatus, the number of coils in, and the thickness of, the sperm cord and the ornamentation of the middle-piece may be sources of key information. By identifying a family-wide pattern in gross spermatophore morphology, this study aims to maximize the information gained from preserved specimens and to increase the efficiency with which such information is collected.

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Appendix 1. *Listed alphabetically by genus and species are the 44 taxa considered here. Each taxon is listed with its taxonomic authority, any additional literature sources used and museum specimens examined and the number of spermatophores from each source. The instances in which two spermatophores from a single specimen were measured are noted. Museum abbreviations are CAS, California Academy of Sciences; CMN, Canada Museum of Nature; FMNH, Field Museum of Natural History; UA, University of Arizona Invertebrate Collection; UMML, University of Miami Marine Laboratory.*

Ameloctopus litoralis Norman, 1992b N=2; *Bathypolypus arcticus* Prosch, 1849 (Muus, 1962) N=1, FMNH 278080 N=1; *Benthoctopus januarii* (Hoyle, 1885) (Toll, 1981) N=3; *Benthoctopus karubar* Norman et al., 1997 N=1; *Benthoctopus macrophallus* Voss & Pearcy, 1990 N=1; *Benthoctopus oregonensis* Voss & Pearcy, 1990 N=1; *Benthoctopus piscatorum* (Verrill, 1879) FMNH 281720 N=1; *Benthoctopus yaquinae* Voss & Pearcy 1990 N=2, FMNH 278308 (two spermatophores measured); 278309; 278311; 278119 N=5; *Benthoctopus* spp. CAS 018763 N=1; FMNH 278066 N=1; *Cistopus* sp. ANSP 6394 N=1; *Eledone caparti* Adam, 1950 UMML 31.1425 N=3; *Eledone cirrhosa* Lamarck, 1798 (Fort, 1937) N=2; *Eledone gaucha* Haimovici, 1988 UMML 31.2177 (two spermatophores measured) N=2; *Eledone moschata* Lamarck, 1799 (Fort, 1941) N=2; *Eledone palari* Lu & Stranks, 1991 N=13, (Norman et al., 1997) N=1; *Enteroctopus dofleini* (Wülker, 1910) (Sasaki, 1929) N=2; *Graneledone pacifica* Voss & Pearcy, 1990 N=2; FMNH uncat. (two spermatophores measured); 281002; 281001; 278061 N=5; *Graneledone verrucosa* (Verrill, 1881) CMN 92522 (two spermatophores measured) N=2; *Octopus aegina* Gray, 1849 CAS 077992 N=2; *Octopus alpheus* Norman, 1992c N=3; *Octopus aspilosomatis* Norman, 1992c N=2; *Octopus australis* Hoyle, 1885 (Stranks & Norman, 1992) N=9; *Octopus berrima* Stranks & Norman, 1992 N=6; *Octopus bimaculatus* Verrill, 1883 (Pickford & McConnaughey, 1949) N=16; *Octopus bimaculoides* Pickford & McConnaughey, 1949 N=4; *Octopus briareus* Robson, 1929 (Pickford, 1945) N=1; *Octopus campbelli* Smith, 1902 (Stranks & Norman, 1992) N=1; *Octopus dierythraeus* Norman, 1992c N=2; *Octopus exannulatus* Norman, 1992a N=3; CAS 077990; 077994 N=4; *Octopus filosus* Howell, 1868 (Alvina, 1965) (as *Octopus hummelincki* Adam, 1936) N=23; *Octopus fitchi* Berry, 1953 UA N=6; *Octopus graptus* Norman, 1992a N=1; *Octopus joubini* Robson, 1929 (Pickford, 1945) N=2; *Octopus macropus* Risso, 1826 (Pickford, 1945) N=1; *Octopus mototi* Norman, 1992a N=2; *Octopus polyzena* Norman, 1992a N=1; *Octopus pyrum* Norman et al., 1997 N=2; *Octopus veligero* Berry, 1953 UA N=1; *Octopus vulgaris* Cuvier, 1797 (Pickford, 1945) N=3; *Octopus* sp. n. near *aculaeatus*, CAS 077987; 077999; 078000 N=6; *Octopus* sp. n. near *abaculus*, CAS 078001; 078002 N=6; *Pareledone charcoti* (Joubin, 1905) UMML 31.2561 N=1, UCMP N=1; *Pareledone* sp., UMML 31.2562 N=1; *Vosseledone charrua* Palacio, 1978 N=2; *Vulcanoctopus hydrothermalis* Gonzalez et al., 1998 FMNH 287365 N=1.

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