CHAPTER 5

Variability in cocoon size in southern African wild silk moths: implications for sustainable harvesting

INTRODUCTION

In addition to the domesticated or mulberry silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), many indigenous wild silk moth species have been utilized for over 2000 years (Peigler 1993). Although *B. mori* silk currently satisfies 95 - 99 % of the demand for commercial silk (Peigler 1993; Scoble 1995), the low volume of wild silk supplies an exclusive niche market where scarcity and naturalness is highly valued.

Southern Africa has two indigenous silk moth species, *Gonometa postica* Walker and *G. rufobrunnea* Aurivillius (Lepidoptera: Lasiocampidae), that produce high quality silk (Nagaraju & Jolly 1988). *Gonometa* silk is slightly coarser than *B. mori* silk, but finer than other wild silk moth species (Hartland-Rowe 1992, Freddi *et al.* 1993), has a natural gold colour and dyes well (Hartland-Rowe 1992). The cocoons of both species are thus considered a valuable natural resource.

Despite similar cocoon characteristics, there are marked differences between the two *Gonometa* species. *G. postica* is polyphagous (hosts include *Acacia erioloba*, *A. tortillis*, *A. mellifera*, *Burkea africana*, *Brachystegia* spp. and the alien, *Prosopis glandulosa*), whereas *G. rufobrunnea* feeds only on *Colophospermum mopane* (Scholtz & Holm 1985; Hartland-Rowe1992). The distributions of the species also differ (Fig. 1) (Pinhey 1975; Hartland-Rowe1992). Male and female adults of *G. postica* have brown fore wings, while those of *G. rufobrunnea* are red (Pinhey 1975). Although the general biologies of both species are reasonably well known (Pinhey 1975; Scholtz & Holm 1985; Hartland-Rowe 1992), the ecology of neither has been studied. For example, the spatial and temporal variation of natural

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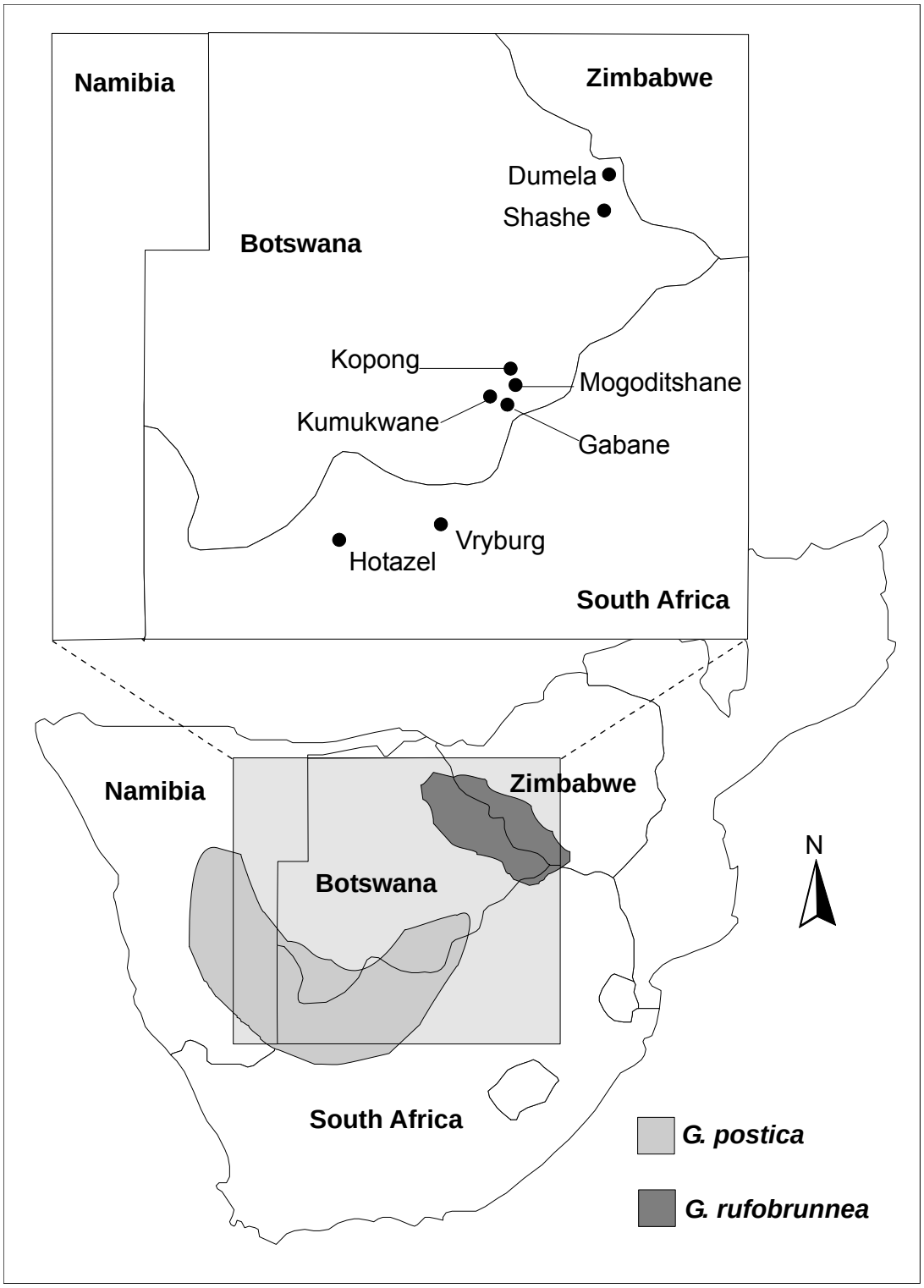

Figure 1. Known distribution ranges of eruptive phases (as from previous published and historic reports, as well as personal observations) of *Gonometa* species in southern Africa, as well as sampled localities.

population sizes, and the impacts of natural enemies, host plant distribution and quality, are not known. Despite this, harvesting of *G. rufobrunnea* has been extensive. Between 1986 and 1987, 430 tonnes of cocoons containing live pupae (here after referred to as 'occupied cocoons') were collected by inhabitants of the Francistown area (Botswana) and sold to Shashe Silk Ltd. (Hartland-Rowe 1992). A decrease in cocoon abundance and a fall in the international silk price coincided with the abrupt end of this enterprise (McGeoch 2000). However, because the population dynamics of the species are unknown, the decline in abundance could not be unequivocally attributed to over harvesting.

Presently, empty cocoons (i.e. from which adults have emerged) are being collected from natural populations of *G. postica* in the North West Province of South Africa. Although this practice may be sustainable, the quality and market value of silk extracted from empty cocoons, which are usually older and with the cocoon surface ruptured by emergence holes (caused by the moth itself or parasitoids), is lower than that from occupied cocoons. There is thus still extensive potential pressure on natural populations of *Gonometa* from harvesting of occupied cocoons, and additional information on the species is required to develop a sustainable harvesting programme.

One component of this information is the identification of *Gonometa* species and sex using cocoons in the field, as well as identifying additional patterns of variability in cocoon size. This not only has implications for the estimation of silk yields, but also for extracting biological information, such as sex ratio and population density, from cocoons in the field. Studying the pupal stage has a number of advantages. Cocoons are sessile, conspicuous and therefore more readily measured and monitored than mobile stages. Cocoons are also the raw material for silk production, and variation in their size is thus important. With as many as 670 cocoons of *G. rufobrunnea* needed to produce one kilogram of raw silk (Hartland-Rowe 1992), variation in cocoon size of naturally harvested populations could markedly affect silk yields. In addition to reported cocoon size sex and species differences (Nagaraju & Jolly 1988; Hartland-Rowe 1992), variability in host-specific populations and geographically separated localities may exist. The frequency of dwarfism (significantly smaller than average cocoons) that has been observed in populations is also unknown. Bivoltinism in these species may also result in cocoon size differences between generations.

Although occupied cocoon mass (OCM) (as a measure of cocoon size) has been shown to be positively correlated with cocoon volume in other Saturniidae (Tripathi *et al.* 1988), using OCM to study size variability has three disadvantages. It requires destructive sampling, cocoon mass is highly influenced by the status of the pupa (live, dead or parasitised), and although pupal (and thus cocoon) mass is often related to fecundity in Lepidoptera species (Wickman & Karlsson 1989, Garcia-Barros 2000), it is not always so (Leather 1988). Cocoon length is proposed as an alternative size measurement that is accurate and practical, and may in fact be a better measure of potential reproductive effort (Leather 1988, although see Robison *et al.* 1998).

The aims of this study were thus to determine: 1) if these *Gonometa* species are significantly sexually dimorphic in cocoon length, width and shape and if sex can be determined using these measurements; 2) whether cocoon length is a suitable surrogate measure for occupied cocoon mass, and can be used to estimate cocoon silk yield; 3) the sex ratios of the species within and between generations and localities; 4) the frequency of observed dwarfism in populations of both *Gonometa* species and whether this varies between generations; 5) whether cocoon length differs between populations on different host plants, between localities and between the first and second generations.

MATERIAL AND METHODS

G. postica was sampled in North-central South Africa (Vryburg and Hotazel) and South-Eastern Botswana (Gabane, Kumukwane, Mogoditshane and Kopong), while *G. rufobrunnea* was sampled from North-Eastern Botswana (Shashe and Dumela) (Fig. 1; Table 1). Sites were selected based on cocoon abundance, with a minimum of 30 first-generation cocoons per site required for site selection. Sampling was standardized by delimiting an approximately rectangular area incorporating 100 trees at each site (from here on referred to as a grid). This was done to compensate for possible tree-density differences between host-plants and between geographically separated sites (see Table 1). Cocoons were found on all above-ground parts of the tree, and occasionally on herbs growing directly beside the tree trunk. Every cocoon on each tree of each grid was counted and its length measured. At least three grids per host plant

were selected. Sampling of grids started in winter when all larvae had pupated to over-winter. Cocoons formed during this period are hereafter referred to as the first generation. Two months after adult emergence in spring, newly formed cocoons were sampled again at the same grids, which are hereafter referred to as the second generation.

Table 1. Localities where both *Gonometa* species were sampled (first and second generation) and associated host-plants. A grid refers to the sample area incorporating 100 trees.

Species	Host plant	Locality	Co-ordinates	No. grids	Grid area (m^2)	#1st	#2nd
G. postica	*Acacia erioloba*	Hotazel	27° 15' S 23° 03' E	1	9750	1	1
		Vryburg	26° 59' S 24° 40' E	2	6726 ± 2*	2	2
	Acacia tortillis	Gabane	24° 37' S 25° 46' E	1	2500	1	1
		Kumukwane	24° 38' S 25° 40' E	1	3105	1	1
		Mogoditshane	24° 34' S 25° 50' E	1	2243	1	0
		Kopong	24° 31' S 25° 48' E	1	1679	1	1
G. rufobrunnea	*Colophospermum mopane*	Shashe	21° 31' S 27° 24' E	3	380 ± 31*	3	3
		Dumela	21° 07' S 27° 32' E	2	444 ± 73*	2	2

* = mean grid area (± S.E.) for a locality where more than one grid was sampled; #1st = number of first generation surveys, conducted from June to August 2000; #2nd = number of second generation surveys, conducted from January to February 2001. The second generation cocoons of *G. postica* at Mogoditshane could not be sampled due to destruction of the habitat.

To establish the relationship between other cocoon size variables, i.e. length, width and mass, at least 50 occupied cocoons (referred to as harvested cocoons) were removed from trees in areas at least 0.5 km away from each grid. Grids closer than 2 km apart, shared the same sample, while separate samples were taken for those more than 2 km apart. Cocoons were collected during sampling of both the first and second generations. The longest axis of a cocoon was taken as length, while width was measured at the widest section of the cocoon perpendicular to its length. Dimension measurements were taken with a digital caliper accurate to 0.01 mm, while mass was determined with an electronic balance accurate to 0.01 g. Simple and multiple regressions of cocoon length and width with mass were done separately for the sexes of each species because of the marked differences between males and females.

To determine the validity of classifying males and females based on cocoon size and shape alone, the shape of approximately 300 harvested first generation cocoons of each species' was quantified by examining the length-width ratio (LWR). Individuals were categorized as dead, parasitised, emerged, or not yet emerged (cocoon occupied). Dwarfs were defined as individuals smaller than approximately two standard deviations of mean male cocoon length. On emergence, adult moths show marked sexual dimorphism (Pinhey 1975). First generation pupae that failed to emerge were sexed (using the two rounded genital scars on abdominal segment eight and nine of females and the single scar on segment nine of males; see Scoble 1995, p. 131-132). As insufficient time had elapsed for second generation emergence only pupae of this generation, falling in the length- and width-overlap range of the first generation, were sexed. However, it is likely that all sex identification errors were determined because no sex identification errors were made outside of the sex-size overlap range.

Analysis

Differences in cocoon size between sexes, species, generations and localities were determined using generalized linear models (maximum likelihood technique) that have no strict normality assumption for the dependent variable (McCullagh & Nelder 1989). This made it possible to simultaneously investigate differences between separate species-sex combinations. Because of the marked sexual dimorphism in both species, male and female cocoon size data were analysed separately. Thus when considering factors such as generation, host-plant (*G. postica* only) and locality, a model explaining cocoon length was built separately for species

and sexes. Only one host species was found per locality, and localities with a specific host species were closer to each other than to those with other host species. This constraint on the sampling design resulted from a shortage of sampling sites where cocoons were sufficiently abundant. Because *G. postica*'s two host plant species were not found at the same localities, the possible effect of host plant and /or locality on cocoon size could not be separated in this study. However, if no consistent differences in cocoon size are found between localities on which the species occur on different host plant species, then the conclusion may be drawn that host plant species does not affect cocoon size.

RESULTS

Both *Gonometa* species were sexually dimorphic with regard to cocoon mass, length, width and colour. The cocoons of *G. postica* are white with brown setae, whereas *G. rufobrunnea* has red cocoons with red setae. Although mean OCM of *G. postica* and *G. rufobrunnea* only differed significantly between females ($F_{3,620}$ = 1629.46; P < 0.001; R^2 = 88.7%), all species-sex combinations were significantly different from each other with regard to length and width ($F_{3,620}$ = 960.90; P < 0.001; R^2 = 82.2% and $F_{3,620}$ = 1034.02; P < 0.001; R^2 = 83.2% respectively) (Table 2).

Table 2. Mean (± S.E.) cocoon mass (occupied), and length and width of male and female cocoons of both *Gonometa* species. Different letters (superscripts) indicate a significant difference of P < 0.05 between means.

Species	Sex	n	Mass (g)	Length (mm)	Width (mm)
G. postica	male	248	2.85 ± 0.02 [a]	36.00 ± 0.11 [a]	16.41 ± 0.05 [a]
	female	227	6.81 ± 0.06 [b]	45.87 ± 0.17 [b]	21.34 ± 0.08 [b]
G. rufobrunnea	male	55	2.72 ± 0.04 [a]	35.17 ± 0.24 [c]	15.81 ± 0.10 [c]
	female	94	5.13 ± 0.07 [c]	41.82 ± 0.21 [d]	19.26 ± 0.13 [d]

Cocoon length explained approximately 45 % and 60 % of the variation in OCM of both *Gonometa* species males and females respectively. Cocoon length generally explained more of the variation in OCM than cocoon width, but together length and width explained 11 % to 18 % more of the variation in mass than length alone (Table 3). When comparing males and females, cocoon length and width, separately and together consistently explained more (7 % to 22 %) of the variation in mass of females than of males (Table 3). These parameters also generally explained more of the variation in mass of *G. rufobrunnea* than of *G. postica* (ranged from 1 % less to 13 % more) (Table 3).

Table 3. R^2 (%) for simple and multiple (corrected R^2) regressions of length and width on occupied cocoon mass of both *Gonometa* species. Each species-sex combination was analysed separately. All relationships were significant at $P < 0.001$.

Species	Sex	n	Length	Width	Length & width
G. postica	male	248	42.2	40.3	59.6
	female	227	55.2	56.2	72.2
G. rufobrunnea	male	55	48.4	48.1	58.3
	female	93	68.2	55.4	80.1

Males and females of *G. postica* were generally longer than those of *G. rufobrunnea* (Fig. 2a & b). Also, the length overlap range between males and females was smaller for *G. postica* (2 %) than *G. rufobrunnea* (17 %) (Fig. 2a & b). When comparing the differences in cocoon length between sexes and between species, sex differences ($F_{1,3969} = 12566.608$, $P < 0.01$) were far greater than between-species differences ($F_{1,3969} = 1265.649$, $P < 0.01$), although both were highly significant (Table 4).

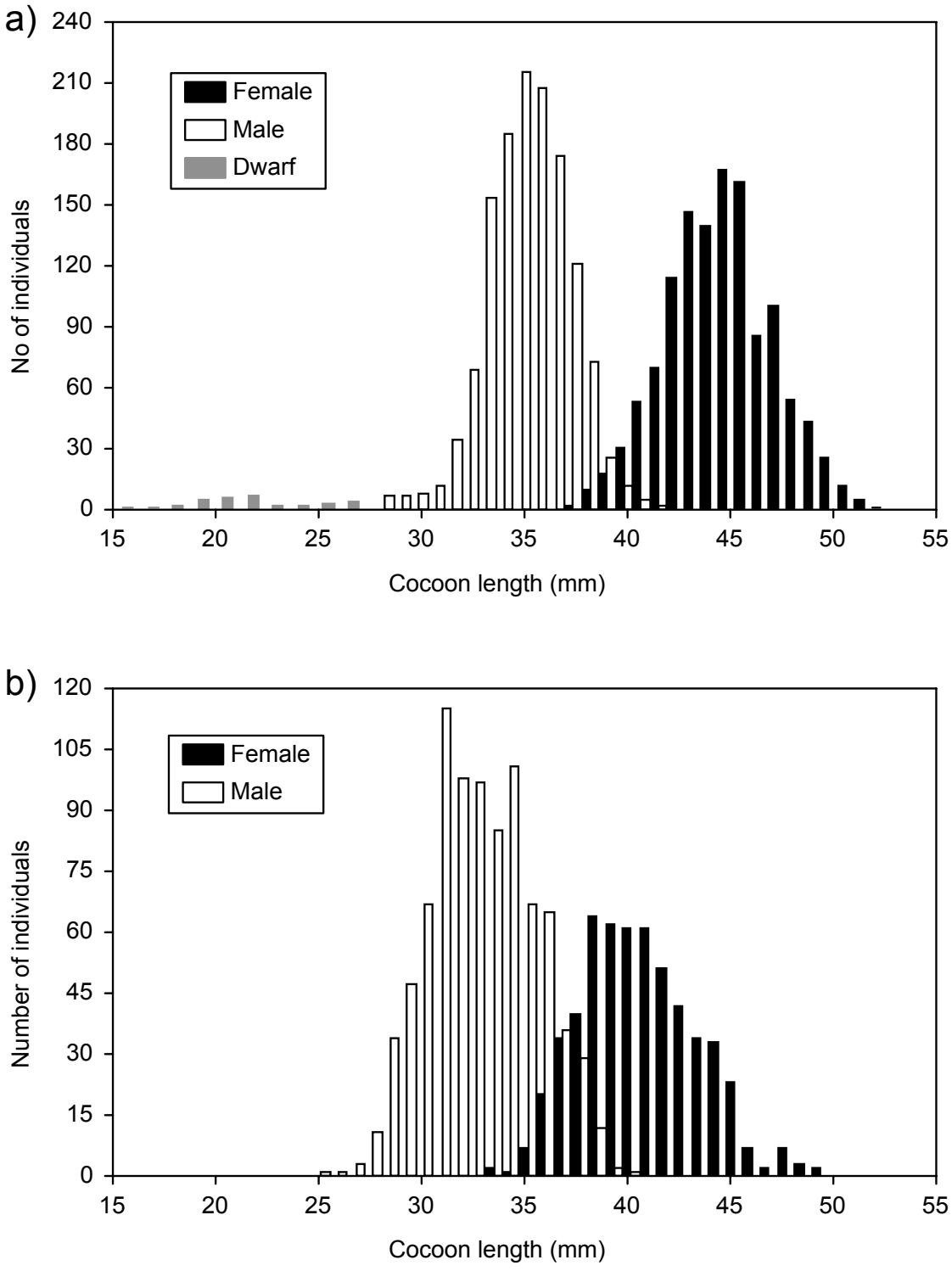

Figure 2. Cocoon length frequency distribution of all field-measured cocoons of a) *Gonometa postica* and b) *G. rufobrunnea*. Note the presence of dwarf individuals for *G. postica*.

Table 4. General linear models of cocoon length on species and sex. Mean (± S.E.) cocoon length differences between *G. postica* and *G. rufobrunnea* and between males and females of both *Gonometa* species are indicated. $P < 0.01$ is denoted by **.

Species	Sex	
	Female	Male
G. postica	44.57 ± 0.07	35.12 ± 0.06
G. rufobrunnea	40.66 ± 0.12	32.94 ± 0.09

Whole model: $R^2 = 79\%$, $F_{2,3969} = 7341.527$**

The length-width ratio (LWR) of males and females of both *Gonometa* species generally had the same range (Fig. 3a & b), but the slopes of the sexes of *G. postica* differed more between each other than those of *G. rufobrunnea* (slopes for the sexes of both species differed significantly at $P < 0.001$ and $P < 0.01$ respectively). Also for both species the rate of increase of LWR with an increase in length was greater for males than for females (Fig. 3a & b). One second-generation *G. postica* female cocoon was malformed (cocoon shape not ovoid) and its length and width (33.67 mm and 15.53 mm respectively) was more typical of a male cocoon than a female. Although this individual fell in the middle of the male LWR against cocoon length scatter, it still emerged successfully as a female and even oviposited a number of eggs. However as a general rule, where length or width overlap occurs, sex identification is still possible because male cocoons are narrower than females at the same length.

The harvested cocoons of *G. postica* had smaller length and width overlap ranges, and a smaller proportion of the population fell in this range compared to *G. rufobrunnea* (Table 5). Also for OCM, *G. postica* had a greater range of no mass overlap between the sexes (males < 3.64 g and females > 3.75 g) than those of *G. rufobrunnea* (males < 3.39 g and females > 3.41 g). Although fewer sex identification errors were made for *G. postica* than *G. rufobrunnea*, when standardised for the proportion of the population in the overlap range, the percentage sex identification errors made was similar for both species (Table 5).

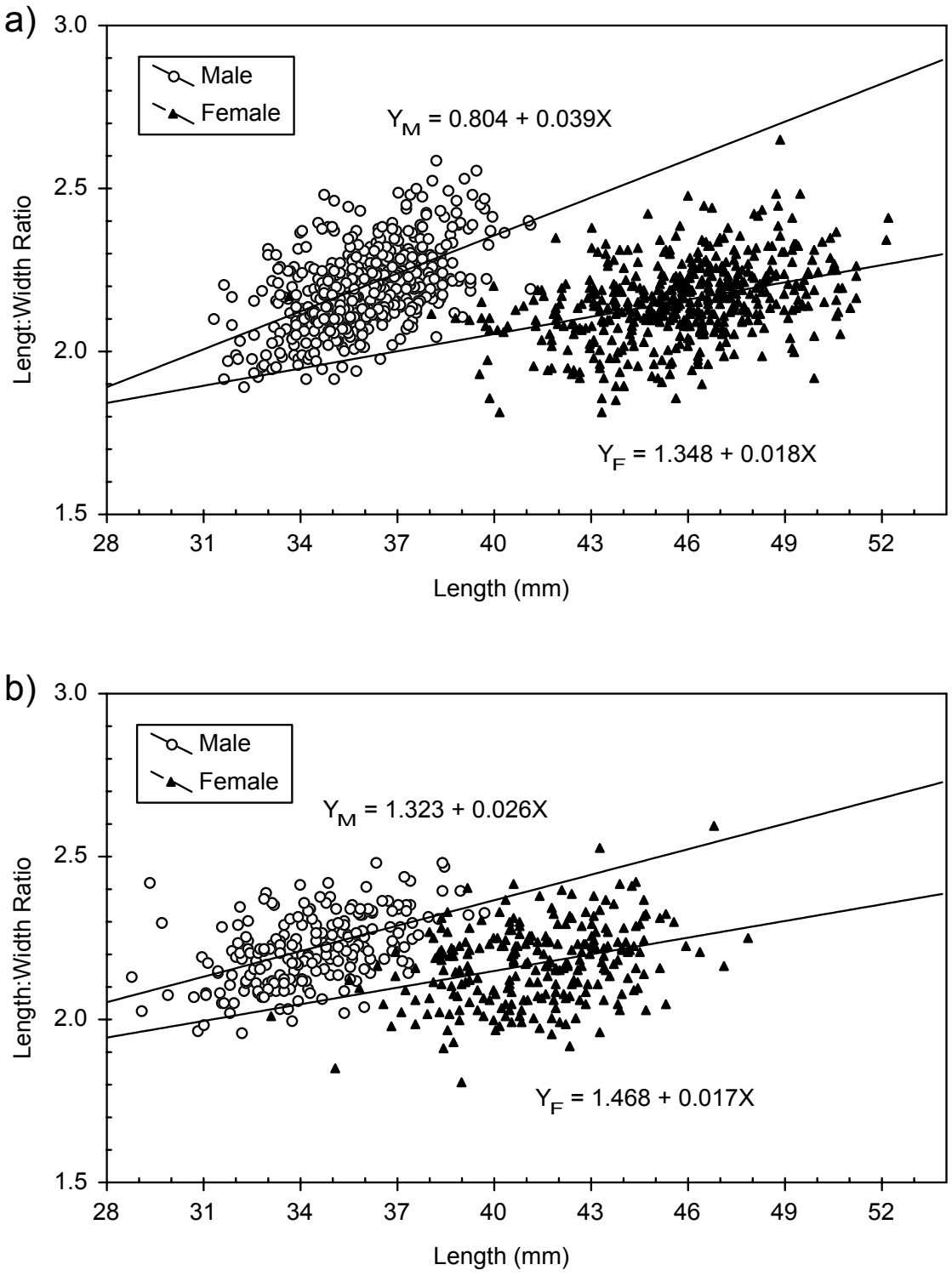

Figure 3. Cocoon length-width ratio against cocoon length and linear regression equations for a) *Gonometa postica* and b) *G. rufobrunnea*.

Table 5. Comparison of the overlap range of male and female cocoons of both *Gonometa* species and subsequent accuracy of sexing.

Species	N	Overlap range (mm)				IDE	% pop.	% IDEOR
		Width	Length	n	% pop.			
G. postica	978	17.50 – 19.00	38.00 – 41.50	20	2.04	3	0.31	15.00
G. rufobrunnea	472	16.40 – 18.50	33.00 – 40.00	79	16.74	10	2.11	12.70

% pop. = percentage of population; IDE = number of identification errors; % IDEOR = percentage of Identification Errors made in Overlap Range.

A sex ratio of 1:1 was expected for both species and this was the case in 19 out of 23 surveys (not 24 due to destruction of a grid, see Table 1). Exceptions included (χ^2 = 1 df): Mogoditshane ($P < 0.05$), Dumela1 and Dumela2 (both $P < 0.001$) first generation surveys, as well as Gabane second generation survey ($P < 0.001$). In all these cases the sex ratio was male biased.

G. postica was the only species with dwarf individuals (Fig. 2a). For this species there was no consistent difference in the frequency of dwarfism found for the first and second generation, but more dwarfs were found on *Acacia erioloba* than on *A. tortillis* (75 % and 25% dwarfs respectively). In only one case, namely first generation cocoons of *G. postica* with *A. tortillis* as host plant, were no dwarfs recorded. The frequency of dwarfism was low and in most cases occurred in approximately 1.5 % of the sampled population. Dwarf cocoons ranged between 15.13 - 27.31 mm in length (n = 32).

When considering each species-sex combination separately there were no significant differences between the lengths of first- and second-generation cocoons, except for *G. rufobrunnea* males where the second generation was longer than the first. In contrast, lengths differed significantly between localities for all four species-sex combinations (Table 6). *G. postica* males and females were the only groupings where host-plant type may potentially affect cocoon size. Despite significant differences between *G. postica* localities, they were not consistent between host specific localities and differed between males and females. The only pattern that emerged was that Gabane and Mogoditshane had the highest mean male and

female cocoon lengths, while Vryburg and Hotazel had the lowest values (Table 7). Comparing localities of *G. rufobrunnea*, Dumela had a highly significant lower mean male and female cocoon length than Shashe.

Table 6. General linear models of cocoon length on generation and locality for each species-sex combination.

Species-sex combinations	R^2	d.f.	F	P	Variable	d.f.	F	P
G. postica	0.06	6	15.082	< 0.001	Generation	1	3.779	0.052
females					Locality	5	17.896	< **0.001**
G. rufobrunnea	0.22	2	79.253	< 0.001	Generation	1	2.587	0.108
females					Locality	1	158.476	< **0.001**
G. postica	0.04	6	9.279	< 0.001	Generation	1	1.486	0.223
males					Locality	5	11.132	< **0.001**
G. rufobrunnea	0.28	2	166.842	< 0.001	Generation	1	9.236	**0.002**
males					Locality	1	333.084	< **0.001**

Table 7. Mean (± S.E.) cocoon lengths of both *Gonometa* species' males and females for generations, localities and host plants. Different letters (superscript) indicate a significance of $P < 0.01$. See Table 6 for analyses.

Category	Type	Cocoon length (mm)			
		Female	n	Male	n
G. postica					
Generation	1st generation	44.62 ± 0.09 [a]	850	35.12 ± 0.07 [a]	826
	2nd generation	44.46 ± 0.12 [a]	390	35.13 ± 0.10 [a]	485
Locality					
A. erioloba	Vryburg	43.84 ± 0.14 [a]	328	34.78 ± 0.11 [a]	268
	Hotazel	44.05 ± 0.14 [ab]	260	34.64 ± 0.12 [a]	242
A. tortillis	Gabane	45.35 ± 0.14 [c]	386	35.61 ± 0.10 [b]	481
	Kumukwane	44.51 ± 0.19 [abd]	145	34.88 ± 0.16 [a]	147
	Mogoditshane	45.44 ± 0.25 [cd]	69	35.25 ± 0.16 [ab]	109
	Kopong	45.05 ± 0.33 [bcd]	52	35.02 ± 0.28 [ab]	64
G. rufobrunnea					
Generation	1st generation	40.66 ± 0.12 [a]	542	32.93 ± 0.09 [a]	846
	2nd generation	40.54 ± 0.37 [a]	14	33.26 ± 0.27 [b]	26
Locality	Shashe	41.79 ± 0.15 [a]	321	34.57 ± 0.12 [a]	339
	Dumela	39.11 ± 0.14 [b]	235	31.89 ± 0.09 [b]	533

DISCUSSION

Marked size differences were found between *G. postica* and *G. rufobrunnea*, with all cocoon size measurements differing significantly between species. These differences, as well as species cocoon colour differences, make species identification in the field based on cocoon morphology possible. Cocoon size differences between sexes were greater for *G. postica* than *G. rufobrunnea*. This is to be expected from allometric scaling of sexually dimorphic species where females are the larger sex (see Fig. 1, Fairbairn 1997). As species become larger so do the intra-specific differences between the sexes, as well as the deviation from the expected isometric scaling constant (Fairbairn 1997).

Sexing cocoons based on shape was found to be acceptable because males were generally narrower (high LWR) than females (low LWR) at the same cocoon length. Simultaneously, male width decreased at a faster rate than female cocoon width with an increase in cocoon length. However, the accuracy of sexing harvested cocoons of both *Gonometa* species was occasionally compromised when males were longer and females shorter than usual. Consequently, for *G. rufobrunnea* more sex identification errors were made. However, standardising the number of identification errors for the proportion of the population, the rate of misidentification was approximately equal for both species. Thus the proportion of the population in this range, and not the size of the length and width overlap range, influenced the number of sex identification errors. Despite these complications in sex identification, both species' cocoons were found to be sexually dimorphic and could be sexed with reasonable confidence in the field (99.7 % accuracy for *G. postica* and 98 % for *G. rufobrunnea*).

Although length-mass regressions of each species-sex combination in this study were significantly positive, the R^2-values were only approximately 50 %. Using both cocoon length and width approximately 15 % more of the variation in OCM was explained. Therefore, although it is possible to estimate silk yield from cocoon length, these estimates will have limited accuracy. There is however no information to date that suggests that cocoon mass would provide more accurate estimates of silk yield. In the single study that examined the latter relationship, no measures of variability were provided (Nagaraju & Jolly 1988). Further research is thus needed to quantify the relationships between pupal mass, cocoon length and silk yield. Based on the results presented here, it is nonetheless possible to use cocoon length

of individuals at a site to determine the size distribution of individuals in the population and to estimate (with a measured degree of accuracy) the potential silk yield of that plot.

Gonometa species from southern Africa were found to have an equal sex ratio, with exceptions being male biased. These exceptions did, however, not occur at the same locality or in both generations, which suggests that they were chance deviations caused by unknown factors in some populations (see for example Jiggins *et al.* 1998, Myers *et al.* 1998).

As dwarfism occurred only in *G. postica* in approximately 1.5 % of the total population on either of the host plant species affected, concerns related to harvesting cocoons from natural populations are apparently unfounded. When dwarfs occur at such low frequencies they should have no effect on the average silk yield per cocoon of harvested natural populations. The cause of dwarfism in *G. postica* or the sex of these individuals is presently unknown. No occupied dwarf cocoons have ever been observed in the field, and it is thought that these cocoons do in fact not contain viable pupae.

Cocoon length variability between populations on different host plant species, from different localities, or of different generations, may also affect the patterns of utilisation, should harvesters select larger cocoons. However, this study showed no significant differences in length between *G. postica* cocoons from its two host plant species between generations. Although locality differences were found for both species, there was much less variation in the cocoon length of *G. postica* between localities than *G. rufobrunnea*. The opposite may have been expected because *G. postica* was found on two host plant species over a wider geographical range than *G. rufobrunnea.* This suggests that host plant species plays little role in determining cocoon size.

The extent of quantitative cocoon size differences between species, sexes, host-plants, localities and generations, as well as their relative importance, has now been described for the first time. The findings presented here thus form the first component of information necessary to estimate silk yields as part of a sustainable utilisation program for harvesting *Gonometa* spp. in southern Africa.

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