Optimal clutch size of the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae)

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Clutch size of the gall wasp *Diplolepis rosae* was studied in galls on *Rosa spp.* in Eastern Hungary and Romania, Transylvania on four dry pastures. We hypothesised that there was an optimal clutch size, and it was controlled by the emerging rate of the gall inducer and the escaping failure of hatched adults. The most frequent clutch size was 25–30 chambers, and there was a second peak around 60 chambers in each frequency distribution. The most common clutch size did not produce the highest proportion of larval emergence to adulthood. Parasitism rate decreased with increasing clutch size, while the proportion of emerged gall wasps increased and the proportion of dead individuals remained constant. The frequency distribution of clutch sizes was independent of parasitism, emerging failure or survival rate of the gall inducer. The escaping failure rate was also independent from the clutch size.

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1. Introduction

Optimal clutch size and the mechanisms influencing it have been studied in several taxonomic groups (Hays & Speakman 1991, Svensson 1995, Monaghan & Nager 1997), including herbivorous insects (Le Masurier 1994, Kagata & Ohgushi 2004). In gall wasps, there are only a few published studies (Weis *et al.* 1983, Kato & Hijii 1993, Freese & Zwölfer 1996). One trade-off determining clutch (gall) size in gall systems is between the fitness of the offspring and reproductive success of the females (Godfray *et al.* 1991). Female reproductive success can be measured by larval survival rate, which is greatly influenced by the size of the gall that the larvae induce (Stille 1984). The rate of gall parasitism by parasitoids influences larval survival and is related to clutch size (Weis *et al.* 1983, Stille 1984, Tabuchi & Amano 2004). In galls consisting of one large single chamber, the walls may be too thick for the parasitoid ovipositor to penetrate (Weis *et al.* 1983). In galls with many chambers, the inner cells are more difficult to reach than outer ones (Ito & Hijii 2004). Hence, gall wasps that produce larger galls may have higher fitness than those that produce smaller ones (Stille 1984). But large galls can be costly. Increased clutch size is associ-

ated with less nutrition per developing larva and may lead to decreased fecundity of adults (Weis *et al.* 1983). Predation by birds also increases with clutch size (Abrahamson & Weis 1997). Larger galls consist of more cells (Brewer & Johnson 1977); thus, the escape route for a newly hatched gall wasp can be longer. Therefore, individuals hatched in larger galls are more likely to get stuck inside the gall (Kato & Hijii 1993). These mechanisms suggest that there may be a trade-off between emergence rate and clutch size. Emergence rate decreases after a given clutch size in *Urophora cardui* (Freese & Zwölfer 1996). Smaller groups of prey may be more exposed to predation than larger ones (Crawley 1992). The rate of infection by many parasitoid species has been shown to decrease with increasing host density (Stiling 1987). One of the main components of the fitness of gall wasp females is the probability of their daughters surviving to adulthood, which is already known to be influenced by the rate of parasitism (Weis *et al.* 1983). However, variables such as the rate of escaping failure could also limit larval survival.

Here we study the clutch size of the rose gall wasp *Diplolepis rosae* to determine whether reproductive success is limited by clutch size specifically through the inability of hatched larvae to escape the gall. Parasitism rate is known to decrease with clutch size but negative effects of clutch size on emergence rate have been rarely reported. A negative relationship between clutch size and emergence rate could also explain the discrepancy between the most frequent clutch size and maximum gall wasp survival. Maximum emergence rate did not occur at the most frequent clutch size in the gall wasp *Dryocosmus kuriphilus* (Kato & Hijii 1993).

The parthenogenetic gall wasp *Diplolepis rosae* induces multi-chambered galls on rose shrubs (*Rosa* spp.). *D. rosae* usually parasitizes *R. canina*, but galls also occur on *R. dumalis*, *R. rubiginosa*, *R. villosa*, *R. sherardi* and *R. rubrifolia* (Stille 1984). Females of *D. rosae* escape from galls in early spring and lay their clutches in new rose buds within one or two months. The new gall finishes its development in late summer and pupae overwinter within the gall. Parasitoid pressure on *D. rosae* galls is high and the inquiline species *Periclistus brandtii* is also fre-

Table 1. Number of *D. rosae* gall wasp adults and parasitoids emerged from galls of *D. rosae* reared under laboratory conditions.

Year	Site1 2001	Site1 2002	Site2 2001	Site2 2002
D. rosae	164	359	44	323
O. mediator	94	235	42	205
T. bedeguaris	82	137	33	48
T. rubi	11	4	1	1
G. stigma	108	120	23	123
P. bedeguaris	7	77	25	46
C. inflexa	66	210	236	62
P. brandtii	1	73	14	13
E. rosae	2	4	6	1
E. urozonus	0	4	0	0
E. vesicularis	7	13	10	1
Reared indiv.	542	1,236	434	823
No. galls	23	56	13	30
No. chambers	842	2,281	576	1,152

quent (László & Tóthmérész 2006). Parasitisation caused high larval loss in *Orthopelma mediator* but the inquiline seemed to have no negative effects (Stille 1984). Escape failure of gall wasps hatched or of parasitoids has not previously been reported in *D. rosae*.

2. Material and methods

This study was carried out on grazed dry grasslands in Eastern Hungary (47°56'N and 20°48'E, 47°19'N and 21°33'E) and in Western Romania, Transylvania (46°47'N and 23°38'E, 46°31'N and 24°34'E) between 2001 and 2004, covering three gall wasp generations. New galls were collected from rose bushes (*Rosa* spp., mainly *R. canina*) in February and March each year and stored in nylon cups under standard laboratory conditions. Old galls were collected in September and stored in nylon cups under similar conditions until dissections. After emergence, the host and the parasitoid species were separated and identified (Table 1) and they were collected until the dissection of galls. We compared escape failure rate of the gall inducer and its parasitoids in the laboratory to those in the field. Galls were dissected two years after the collecting date (new galls) or directly after collecting (old galls). We

Table 2. Number of dead gall wasp and parasitoid individuals in galls of *D. rosae* reared under laboratory conditions and in galls collected in the field after the emergence of wasps and parasitoids.

| Year | Laboratory | | Field | | | | |
	Site1 2001	Site1 2002	Site2 2001	Site2 2002	Site2 2004	Site3 2004	Site4 2004
D. rosae	39	244	105	75	45	29	145
O. mediator	37	136	24	53	37	13	144
T. bedeguaris	13	14	3	5	15	2	12
T. rubi	2	4	0	1	0	4	4
G. stigma	10	19	10	8	6	4	3
P. bedeguaris	1	13	1	1	16	12	10
C. inflexa	34	48	5	12	2	13	13
P. brandtii	0	19	0	3	7	10	19
E. rosae	0	3	0	0	4	0	1
E. urozonus	0	0	0	0	0	1	0
E. vesicularis	0	2	0	1	0	1	3
Dead larvae	10	99	15	41	78	52	63
Dead pupae	0	24	0	1	5	37	4
Dead indiv.	146	625	163	201	215	178	412
No. galls	23	56	13	30	29	29	41
No. chambers	842	2,281	576	1,152	1,032	1,151	1,551

made 5 mm width dissections using a deep-frame fret saw. All dead larvae, pupae and adults, which failed to emerge (Table 2), were separated and dead adults were identified. We used old galls (from which adult gall wasps and parasitoids emerged under natural (field) conditions) to test whether the laboratory conditions affected the emerging success of adult insects. The effect of laboratory condition was studied only in a fraction of unemerged wasps. After dissections we counted all gall cells.

2.1. Statistical analyses

We collected a total number of 221 galls, from which 122 were new (induced in the year before collecting), and 99 were old (induced with two or more years before collecting). Statistical analysis was carried out only on 207 galls, because we excluded data from extremely large galls (>100 chambers, $n = 14$ galls), which were outliers in all tests and would have biased clutch size distributions. Of the 207 galls analysed, 112 were new and 95 were old.

Differences of data sets were tested by Kruskal-Wallis test and Mann-Whitney U-test. Correlations between variables were tested with Spearman rank analysis. Because of the over-dispersion of the number of emerged adults, dead larvae, pupae and adults (Table 3), we used generalised linear models with Poisson and quasi Poisson error distribution to describe the effects of increasing gall cell number on the emergence of adult gall wasps and parasitoids and on the emerging failure of gall inhabitants. Statistical analyses were carried out with the R language and environment (R 2.3.1, R Development Core Team 2005).

3. Results

No significant differences were revealed in the number of gall chambers among the four sampling sites (Kruskal-Wallis test: $n=207$, $df=3$, $\chi^2=0.43$, $p=0.93$) and the three sampling years (Kruskal-Wallis test: $n=207$, $df=2$, $\chi^2=0.72$, $p=0.69$). The number of emerged gall-inducers did not differ by sampling site (Mann-Whitney *U-test*: $n=112$, $U=1340.5$, $p=0.58$) or by sampling year (Mann-Whitney *U-test*: $n=112$,

Table 3. Numbers of emerged and dead *D. rosae* and parasitoid individuals, their proportions according to mean clutch size after dissections of galls of *D. rosae*, reared under laboratory conditions and in galls collected in the field after the emergence of wasps and parasitoids.

		Laboratory				Field		
	Year	Site1 2001	Site1 2002	Site2 2001	Site2 2002	Site2 2004	Site3 2004	Site4 2004
	No. galls	23	56	13	30	29	29	41
	No. cham.	842	2,281	576	1,152	1,032	1,151	1,551
	Mean no. cham	36.59	41.47	44.40	38.40	35.58	39.68	37.83
Emerged	*D. rosae*	164	359	44	323	–	–	–
	parasitoids	378	877	390	500	–	–	–
	total	542	1236	434	823	–	–	–
Dead	*D. rosae*	39	244	105	75	45	29	145
	parasitoids	97	258	43	84	87	60	209
	larvae, pupae	10	123	15	42	83	89	67
	total	271	625	163	201	215	178	421
Emerged	% *D. rosae*	8.86	12.05	9.97	17.85	–	–	–
	% parasit.	39.61	45.54	56.19	52.97	–	–	–
	% total	48.47	57.59	59.65	70.82	–	–	–
Dead	% *D. rosae*	7.20	8.42	23.41	3.48	6.35	1.69	6.73
	% parasit.	10.76	10.89	9.03	10.51	9.60	5.68	12.92
	% larvae, pupae	1.21	5.9	3.21	6.54	5.38	12.99	4.20
	% total	19.17	25.21	35.73	20.53	21.33	20.37	23.88
Total	%	67.64	82.80	95.38	91.35	–	–	–

U=1197.5, p=0.38). The number of dead adults did not differ significantly among the four sampling sites (Kruskal-Wallis test: n=207, df=3, χ^2=2.32, p=0.5) and three years (Kruskal-Wallis test: n=207, df=2, χ^2=1.02, p=0.59). Number of total adult parasitoids (emerged and dead) did not differ significantly between the two sampling sites, where there were emerged adults (Mann-Whitney *U-test*: n=112, U=1323, p=0.54) and sampling years (Mann-Whitney *U-test*: n=112, U=1361.5, p=0.82). Based on these results, we pooled data from sampling sites and years. Therefore, galls were analysed as belonging to a single data batch.

The comparison of escape failure rate in the laboratory and that in the field showed no significant differences. Similar numbers of adults failed to escape from galls reared in the laboratory and from galls developing under field conditions (Mann-Whitney *U-test*: n=207, U=5122, p=0.64).

The shape of frequency distribution of clutch sizes was right-skewed (Fig. 1). Galls formed by 21–30 chambers (n=47, 22.7%) were the most frequent, followed by those containing 11–20 chambers (n=37, 17.8%) and by galls formed by 1–10 chambers (n=33, 15.9%). Galls containing 31–40 chambers constituted only 14% (n=29) of the whole gall sample (n=207). Galls with more than 40 chambers formed only 29.4% (n=61), while those below 40 chambers constituted 70.5% (n=146) of the whole sample (Fig. 1a). The frequency distribution of clutch sizes, with a peak at 25–30 chambers and a second, but lower at 60 chambers, was remarkably similar across sampling sites and years (Fig. 1b).

The average emergence rate of newly hatched gall wasp adults was 12.2% and 8.2% of gall wasps were found dead in galls after dissections. The average parasitoid infection rate was 58.5%; from this percentage 48.6% escaped from galls, while 9.9% did not emerge. Finally, 5.6% of the remaining gall cells contained dead larvae and pupae (Table 3, Fig. 2). The remaining 15.5% probably was lost due to parasitoid feeding. A parasitoid larva can consume more than one host larva while it chews itself from one chamber to another (Claridge & Askew 1960).

A total of 3035 individuals were reared from 122 galls containing 4851 chambers (Table 1). From this total, 890 belonged to the gall inducer *D. rosae*. The most frequent parasitoid of

D. rosae was the ichneumonid *O. mediator*, of which 576 individuals emerged, followed by the torymids *Glyphomerus stigma* (374 individuals) and *Torymus bedeguaris* (300 individuals). *Pteromalus bedeguaris*, also a parasitoid of *D. rosae*, was less frequent with only 155 individuals. The presence of the inquiline *P. brandtii* did not reach even 5% of the total reared individuals (101 adults, 3.3%), while its most frequent parasitoid, *Caenacis inflexa*, reached 18.9% (574 adults). Other parasitoids like *Eurytoma rosae*, *Eupelmus urozonus* and *Eupelmus vesicularis* were rare (1.6%, 48 adults). Only 17 adults represented the third torymid parasitoid *Torymus rubi*. Hatched adults emerged from 64.4% of the total number of chambers (Table 1); the rest were stuck and dead in the galls.

After dissections we counted a total of 1,940 dead individuals in 221 galls containing 8,585 chambers (Table 2). Dead individuals, which either died during larval development or in the pupal stage, or could not escape from galls, were found in 22.6% of the chambers (Table 2). From the total number of dead individuals 682 (35.2%) were gall inducers. *O. mediator* was the most frequent parasitoid, represented by 444 individuals (22.9%), whereas the frequency was below 5% for each of all other parasitoids. It is interesting that *P. brandtii* was represented only by 58 (2.9%) individuals, while its major parasitoid, *C. inflexa* by 127 (6.5%). Other parasitoids did not reach even 1%. Dead larvae (n=358) made up 18.5%, and dead pupae (n=71) made up 3.7% of the total number of dead individuals.

The proportion of *D. rosae* adults emerging from the gall was positively related to clutch size (Spearman's correlation: n=112, r_s=0.32, p=0.001), whereas the proportion of parasitoids emerging from the gall was negatively related to clutch size (n=112, r_s=−0.29, p<0.01). The proportion of all dead individuals (both *D. rosae* and parasitoids) remaining in the gall did not correlate with clutch size (n=207, r_s=0.05, p>0.05). After using a Bonferroni correction on the tests per-
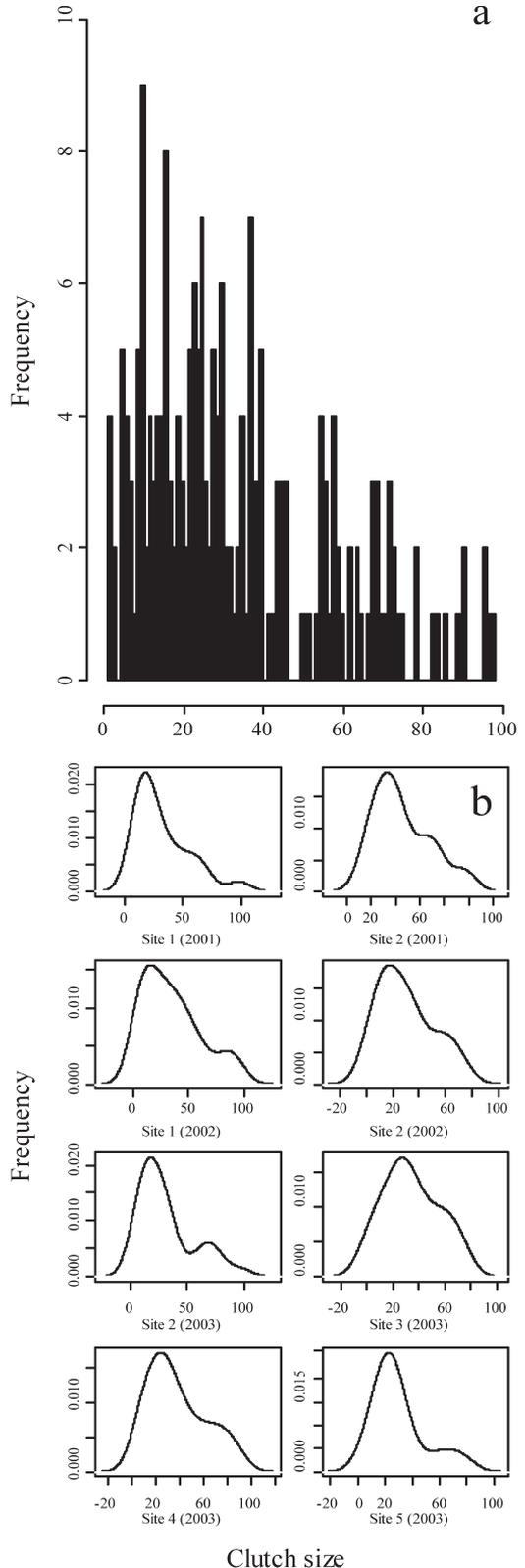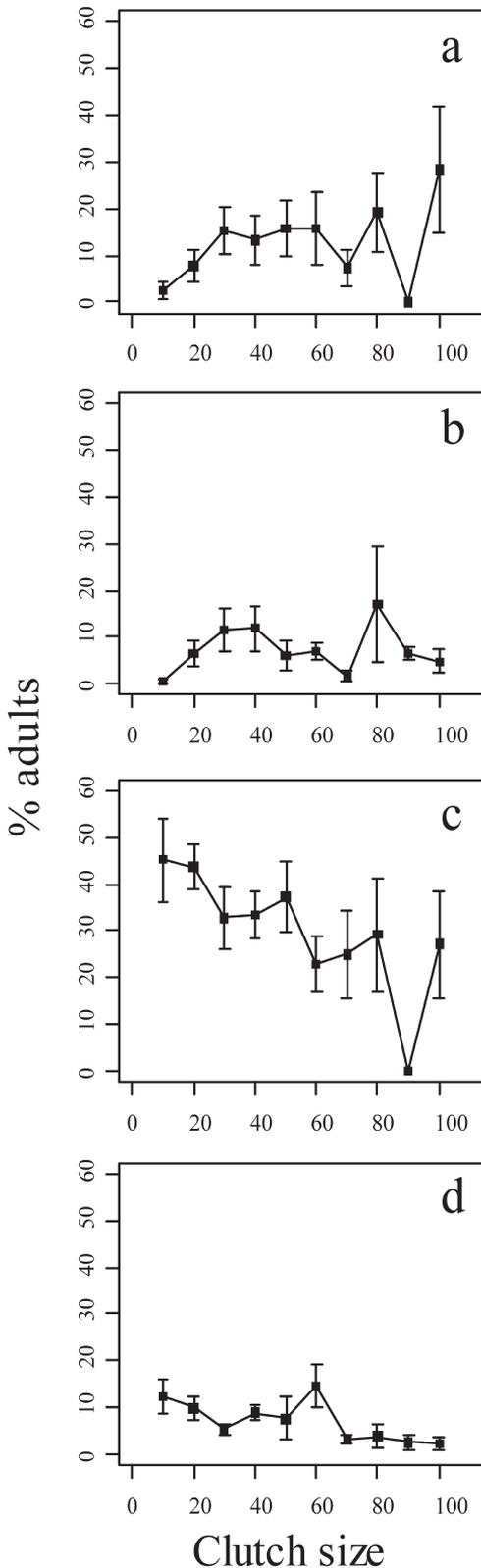

Fig. 1. Clutch size frequency for all tested galls (n=207) (a) and density plots for clutch size frequencies for galls from separate study sites and years (b).

Fig. 2. Mean percentage ± *SE* of emerged (a) and dead (b) gall inducers (*D. rosae*) and emerged (c) and dead (d) parasitoids per gall as a function of clutch size (*n*=112).

formed, all correlations remain significant on a 0.05 level, except the last one. These results show that the proportion of *D. rosae* emerged correlated positively with the clutch size, whereas the proportion of parasitoids emerged correlated negatively with the clutch size. The proportion of dead individuals shows no clear tendency (Fig. 2).

There were no correlations between clutch size frequency and the number of emerged gall inducers (Spearman's correlation: $n=112$, $r_s=-0.16$, $p=0.08$, Bonferroni-corrected $p=0.24$) or the number of total parasitoids ($n=112$, $r_s=-0.14$, $p=0.13$, Bonferroni-corrected $p=0.39$), whereas the total number of dead individuals showed a significant negative correlation with gall frequency ($n=112$, $r_s=-0.26$, $p=0.004$, after Bonferroni correction $p=0.01$). There were also no correlations between either the survival rate of gall inducers ($n=112$, $r_s=-0.11$, $p=0.21$), or the rate of total number of dead individuals individuals ($n=112$, $r_s=0.00$, $p=0.99$) or the rate of total parasitism ($n=112$, $r_s=0.10$, $p=0.25$) and the frequency of clutch sizes. Using GLM with Poisson and quasi Poisson distributions provided similar results, because only the total number of dead individuals showed no significant relationship with clutch size frequency (Table 4).

4. Discussion

Clutch sizes of *D. rosae* showed a characteristic frequency distribution across four sampling sites and three years, i.e., galls with fewer chambers were more common than galls with more chambers. This suggests that there may be a factor limiting the number of chambers per gall. Galls formed by 25–30 chambers represent the most frequent clutch size. Although there was a second peak in the frequency distribution (at around 60 chambers), it is unclear what benefits could be associated with such a second peak. One possibility

Table 4. Results of general linear models testing the effect of clutch size and its frequency on the number of emerged gall inducers, total number of dead individuals and total number of parasitoids.

Clutch size Dependent variables	Error distribution	Disp. parameter	t	p
Emerged *D. rosae*	quasi Poisson	13.13	3.40	<0.001
Total parasitoids	quasi Poisson	13.13	3.49	<0.001
Total no. of dead individuals	quasi Poisson	13.13	3.64	<0.001
Rate emerged *D. rosae*	quasi Poisson	15.16	2.09	0.03
Rate total parasitoids	quasi Poisson	15.16	–0.50	0.61
Rate total no. of dead individuals	quasi Poisson	15.16	–3.16	0.002

Frequency Dependent variables	Error distribution	Disp. parameter	z	p
Emerged *D. rosae*	Poisson	0.99	–1.79	0.07
Total parasitoids	Poisson	0.99	–1.84	0.06
Total no. of dead individuals	Poisson	0.99	–1.20	0.22
Rate emerged *D. rosae*	Poisson	1.09	–1.66	0.09
Rate total parasitoids	Poisson	1.09	–0.77	0.43
Rate total no. of dead individuals	Poisson	1.09	1.06	0.28

is that larger galls may be less frequently parasitised, as suggested by a negative correlation between clutch size and number of parasitoids (Fig. 2). However, this characteristic frequency distribution is not related to the emergence rate of the gall inducer, parasitism or escaping failure of hatched adults from galls.

For the galls of *D. rosae*, the most common clutch size was different from the highest emergence rate producing gall size. We found that larger galls showed higher emergence rates which were represented in smaller numbers than those with fewer chambers. To explain the lower number of those *D. rosae* galls, we expected higher escape failure of hatched adults with increasing clutch size. Escaping failure of cynipid gall inducers was reported for *Andricus quercuscalicis* within a large percentage of galls (10–15%) but cause is not known (Hails & Crawley 1991, 1992). Emerging failure of adults of *D. rosae* showed no relationship with clutch size frequency (Table 4). However, some factors potentially controlling the frequency of clutch sizes in *D. rosae*, are yet to be tested. Controlling factors can be the bird predation, as it is known for *Eurosta solidaginis* (Abrahamson & Weis 1997) or decreased breeding success as in *Asteromyia carbonifera* (Weis *et al.* 1983).

For the gall inducing cecidomyid *A. carboni-*

fera, parasitoid attack shows decrease with increasing clutch size (Weis *et al.* 1983), while in the case of the galler fly *E. solidaginis* (Abrahamson & Weis 1997) the risk of predation by birds increases with increasing clutch size. Bird predation was reported, but not investigated in the case of a single chambered oak gall, *A. quercuscalicis* (Hails & Crawley 1991, 1992). For the gall inducing tephritid fly *U. cardui*, the same positive interaction was found between growing clutch size and parasitoid induced mortality of galler larvae (Freese & Zwölfer 1996). In an earlier study by Stille (1984), it was noticed that as the clutch size of *D. rosae* increased, the rate of parasitism decreased. The results in our study were similar (Table 4, Fig. 2).

Galls with a higher clutch size (or larger galls) are less common than galls with smaller clutches in *A. carbonifera* (Weis *et al.* 1983). With increasing clutch size, the fecundity of offsprings of *A. carbonifera* decreases, thus it is not remunerative to lay a maximal egg load (Weis *et al.* 1983). For the cynipid *D. kuriphilus*, the frequency of smaller clutch sizes was higher than the frequency of bigger ones, and mean emergence proportion showed an increase with increasing clutch size, even if it is not obvious in all cases (Ito & Hijii 2004). Also for *U. cardui*, smaller clutch sizes were more frequent than larger ones

(Freese & Zwölfer 1996). The frequency distribution of clutch sizes of *D. rosae* is in concordance with those found in other galling systems. To gain a more general view of the background, which determines this distribution, we have to study the change of fecundity of females, internal gall structure and bird predation.

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