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EVOLUTIONARY RELATIONSHIPS AMONG FOOD HABIT, LOSS OF FLIGHT, AND REPRODUCTIVE TRAITS: LIFE-HISTORY EVOLUTION IN THE SILPHINAE (COLEOPTERA: SILPHIDAE)

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Flightlessness in insects is generally thought to have evolved due to changes in habitat environment or habitat isolation. Loss of flight may have changed reproductive traits in insects, but very few attempts have been made to assess evolutionary relationships between flight and reproductive traits in a group of related species. We elucidated the evolutionary history of flight loss and its relationship to evolution in food habit, relative reproductive investment, and egg size in the Silphinae (Coleoptera: Silphidae). Most flight-capable species in this group feed primarily on vertebrate carcasses, whereas flightless or flight-dimorphic species feed primarily on soil invertebrates. Ancestral state reconstruction based on our newly constructed molecular phylogenetic tree implied that flight muscle degeneration occurred twice in association with food habit changes from necrophagy to predatory, suggesting that flight loss could evolve independently from changes in the environmental circumstances per se. We found that total egg production increased with flight loss. We also found that egg size increased with decreased egg number following food habit changes in the lineage leading to predaceous species, suggesting that selection for larger larvae intensified with the food habit change. This correlated evolution has shaped diverse life-history patterns among extant species of Silphinae.

KEY WORDS: **Egg production, egg size, flight muscle, necrophagous, ovariole number, predaceous.**

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Resources acquired by an individual organism are divided among three basic functions: somatic maintenance, growth, and reproduction (Winkler and Wallin 1987; Fox and Czesak 2000). Because an organism has a limited amount of resources available, there are trade-offs among these functions (e.g., James 1974). Optimum energy allocation of resources at a given point in time can vary depending on the morphology, physiology, lifestyle, and habitat of the organism. Phenotypic variation in energy allocation can cause complicated phenotypic correlation patterns within species (Van Noordwijk and de Jong 1986; Stearns 1992; Roff 2002). Thus, a wide range of life history and resource allocation patterns can be observed within and among species. Many theoretical studies have addressed the issue of trade-offs in life-history traits (see Roff 2002).

Many extant insect species have lost flight capabilities (Roff 1990; Wagner and Liebherr 1992; Roff 1994). Roff (1986) noted the possible evolutionary sequence to flightlessness in insects, expressed as an initial decrease in dispersal frequency, followed by flight muscle degeneration, and wing degeneration in the final stages. In general, flightlessness in insects can be induced by changes to a habitat that is less spatiotemporally variable, more predictable, less severe, or more isolated (Harrison 1980; Roff 1990; Wagner and Liebherr 1992).

Production and maintenance of the flight apparatus and flight behavior itself can reduce the allocation of resources to reproductive investment because these functions are energetically very expensive (Roff 1991; Lovei and Sunderland 1996; Zera and Denno 1997). Phenotypic correlations between flight traits and relative reproductive investment have been observed in many flight polymorphic species belonging to various taxa, indicating the presence of trade-offs between flight and reproduction (Roff and Fairbairn 1991; Zera and Denno 1997). It is also likely that interspecific phenotypic correlations, that is, macroevolutionary trade-offs (sensu Stearns 1992), exist between flight traits and relative reproductive investment. However, very few attempts have been made to assess macroevolutionary trade-offs between flight and reproductive traits.

Progeny that hatch from larger eggs are likely to have higher juvenile survivorship because they can better withstand environmental stresses such as starvation and desiccation, are superior in larval competition, and have a large range of food sizes (Winkler and Wallin 1987; Wallin et al. 1992; Fox 1994; Fox and Czesak 2000; Marshall et al. 2006). Optimum egg size can be strongly influenced by characteristics of the larval habitat, which can affect the relationship between egg size and larval survivorship (Smith and Fretwell 1974; Parker and Begon 1986; Bernardo 1996; Fox and Czesak 2000; Marshall et al. 2006). It is often the case that flightless morphs with higher total egg production lay more eggs than conspecific flight-capable morphs, although egg size is similar between the two morphs (e.g., Tanaka 1993; Zera and Denno 1997; Hanski et al. 2006). However, if flight ability of an adult determines larval habitat, variation in flight ability might result in variation in selection for egg size (Montague et al. 1981; Zera and Denno 1997; Jonsson 2003).

The family Silphidae (Coleoptera) is composed of two subfamilies: Silphinae and Nicrophorinae. Whereas flightless individuals have never been found in the Nicrophorinae, the Silphinae include some flight dimorphic species and completely flightless species (Ikeda et al. 2007). The adults of most flight-capable silphine species feed mainly on vertebrate carcasses (necrophagy), whereas adults of flightless or flight-dimorphic silphine species feed mainly on soil invertebrates (predatory; Ikeda et al. 2007). If flightlessness is the derived state and necrophagy is the ancestral state, then the evolutionary change to flightlessness may have occurred in association with the change to a predatory lifestyle. Flight-capable adults can generally find predictable soil invertebrate resources, as well as unpredictable vertebrate carcass resources. In contrast, it is difficult for flightless adults to find vertebrate carcasses. Therefore, we hypothesize that the change from a necrophagous to a predatory lifestyle preceded the loss of flight. If this is true, a species' own evolutionary change in food habit would induce flight loss, independently of changes in the environmental circumstances.

Silphine beetles are semelparous, and females lay eggs on or in soil (Balduf 1935; Kurosa 1995; Harusawa 1996; H. Ikeda unpubl. data). A female continues to deposit eggs for up to 2 months, with her ovarioles developing asynchronously. In the phylogeny of the Silphinae, the loss of flight could result in increased resource allocation to total egg production; thus, relative investment to egg production would be higher in flightless species than in flight-capable extant species. Food habits of larvae are thought to correspond to those of adults in the Silphinae. In some flight-capable species, adults oviposit near carcasses or larvae feed on vertebrate carcasses (Sikes 2005; H. Ikeda unpubl. data). In contrast, because flightless adults have difficulty searching for and ovipositing near vertebrate carcasses, their larvae are unlikely to feed mainly on vertebrate carcasses. In fact, larvae fed only dipteran larvae or earthworms develop into adults in some flightless *Silpha* species (H. Ikeda unpubl. data). In predaceous species, larger larvae may have an advantage in searching for prey, tolerating periods of starvation, and handling larger prey. Therefore, the evolutionary change to a predatory lifestyle and flightlessness could increase egg size, which would thereby be larger in flightless species than in flight-capable extant species.

Here, we conducted phylogenetic reconstruction and independent contrast analyses on flight capability, food habit, and reproductive traits to elucidate evolutionary changes of these

Table 1. List of species, their abbreviations, and GenBank accession numbers used in this study.

Taxon	Abbreviations	Accession number			
		16S	28S	Wg	Pepck
Ingroup					
Silphidae: Silphinae					
Oxelytrum discicolle	*Oed*	AB285552	AB285584	AB285647	AB285615
Diamesus osculans	*Dio*	AB285554	AB285586	AB285649	AB285617
Necrodes littoralis	*Ndl*	AB285536	AB285568	AB285631	AB285599
Nd. nigricornis	*Ndn*	AB285544	AB285576	AB285639	AB285607
Thanatophilus rugosus	*Thr*	AB285546	AB285578	AB285641	AB285609
T. sinuatus	*Ths*	AB285548	AB285580	AB285643	AB285611
Oiceoptoma nigropunctatum	*Oin*	AB285540	AB285572	AB285635	AB285603
Oi. subrufum	*Ois*	AB285537	AB285569	AB285632	AB285600
Oi. thoracicum	*Oit*	AB285549	AB285581	AB285644	AB285612
Necrophila americana	*Npa*	AB285543	AB285575	AB285638	AB285606
Chrysosilpha chloroptera	*Chc*	AB285553	AB285585	AB285648	AB285616
Calosilpha brunnicollis	*Cab*	AB285550	AB285582	AB285645	AB285613
Ca. kurosawai	*Cak*	AB285551	AB285583	AB285646	AB285614
Eusilpha jakowlewi	*Euk*	AB285547	AB285579	AB285642	AB285610
E. japonica	*Eup*	AB285539	AB285571	AB285634	AB285602
Dendroxena sexcarinata	*Des*	AB285535	AB285567	AB285630	AB285598
Phosphuga atrata	*Pha*	AB285541	AB285573	AB285636	AB285604
Silpha tristis	*Sit*	AB285542	AB285574	AB285637	AB285605
S. perforata	*Sip*	AB285534	AB285566	AB285629	AB285597
S. longicornis	*Sil*	AB285538	AB285570	AB285633	AB285601
S. imitator	*Sii*	AB285545	AB285577	AB285640	AB285608
Outgroup					
Silphidae: Nicrophorinae					
Ptomascopus morio		AB285559	AB285591	AB285654	AB285622
Nicrophorus concolor		AB285555	AB285587	AB285650	AB285618
Ni. investigator		AB285558	AB285590	AB285653	AB285621
Ni. tomentosus		AB285561	AB285593	AB285656	AB285624
Ni. maculifrons		AB285556	AB285588	AB285651	AB285619
Ni. quadripunctatus		AB285557	AB285589	AB285652	AB285620
Ni. japonicus		AB285560	AB285592	AB285655	AB285623
Ni. orbicollis		AB285562	AB285594	AB285657	AB285625
Staphylinidae					
Oxyporus niger		AB285563	AB285595	AB285658	AB285626
Stenus alienus		AB285565	None	AB285659	AB285628
Agelosus carinatus		AB285564	AB285596	None	AB285627

traits and to test whether correlated evolution contributed to the macroevolutionary trade-offs shown in the extant species. We constructed a molecular phylogenetic tree for the Silphinae because a previous tree (Dobler and Müller 2000) had some nodes with low probability. We tested the following predictions: (1)An evolutionary change from a necrophagous to a predatory lifestyle resulted in loss of flight; (2) Flight loss resulted in selection for increased allocation of resources to total egg production; and (3) The change to a predatory lifestyle resulted in selection for increased egg size.

Materials and Methods

TAXON SAMPLING AND DNA SEQUENCE DATA

We used 21 silphine species from 12 genera (Table 1) collected at 78 sites (73 forests, three riversides, and two rice paddy fields). These genera covered all major ingroup lineages inferred from the rough topology shown by Sikes et al. (2005). Outgroup species were chosen from the subfamily Nicrophorinae (two genera/eight species) and the family Staphylinidae (three genera/three species), two species that are closely related to Silphidae (*Oxyporus niger*

Table 2. Primers used to amplify and sequence gene regions analyzed in this study.

Gene	Primer	Sequence (5′ → 3′)	Direction*	Reference
16S	LR-J-12887	CTC CGG TTT GAA CTC AGA TCA	F	Simon et al. (1994)
	LR-N-13398	CGC CTG TTT ATC AAA AAC AT	R	Simon et al. (1994)
	16SL	ATT CTA AAT YYA WNG CAC TAW TCT GCC AAA	F	Hosoya and Araya (2005)
	16SAH	YGC CTG TTT AWY AAA AAC ATG	R	Hosoya et al. (2001)[2]
	16Sscal[1]	TTT AAA GAT AGA AAC CAA CCT GGC TCA	F	Present study
	16Sscal2[1]	GTA AAA TWT TAA AGG TCG AAC AGA	F	Present study
28S	Rd3.2a2	AGT ACG TGA AAC CGT TCA GGG G	F	Whiting (2002)[2]
	Rd5b	CCA CAG CGC CAG TTC TGC TTA C	R	Whiting (2002)
	28Ssste[1]	TCT TGA AAC ACG GAC CAA GGA GTC TAG CAT	F	Present study
	28Saste[1]	ATA GTT CAC CAT CTT TCG GGT CCC AGC AT	R	Present study
	28Sscal[1]	TCG GCG ACG CTA TAG CTT T	F	Present study
	28Sacal[1]	TTC ACT TTC ATT TCG CCA GTA GGT TT	R	Present study
Wg	LEPWG1	GAR TGY AAR TGY CAY GGY ATG TCT GG	F	Loxdale and Lushai (1998)
	ModLEPWG2	ACT ICG CAR CAC CAR TGG AAT GTR CA	R	Loxdale and Lushai (1998)
	Wgs	GAG TGT AAG TGT CAT GGT ATG TCT GG	F	Present study
	Wga	CGC AGC ACC AGT GGA ATG T	R	Present study
	Wgnics	GTC ATC GGB GAC AAC CTS AAG GAC C	F	Present study
	Wgnica	AGG TCG CAG CCG TCA ACG CCG AT	R	Present study
	Wgsils[1]	TGG ATG CGT TTR CCA CCR TT	F	Present study
	Wgsila[1]	TTG CAC CGY TCG ACG ACG AC	R	Present study
Pepck	18.5dF	TGT GGN AAR ACC AAY YTG GCC ATG	F	Loxdale and Lushai (1998)
	22.5drc	GAA CCA RTT RAC RTG RAA GAT C	R	Loxdale and Lushai (1998)
	Peps	GGA GAT GAT ATY GCT TGG ATG	F	Present study
	Pepa	GCW GCA GCR GTA GCT TCA CT	R	Present study
	Peps2	GGA GAT GAT ATT GCT TGG ATG	F	Present study
	Pepa2	GCA GCA GCG GTA GCT TCA CT	R	Present study
	Pepnics	GAC GAC ATC GCY TGG ATG CGY TT	F	Present study
	Pepnica	GCG GCD GTD GCY TCG CT	R	Present study
	Pepnecs[1]	GGA TTC TTC GGT GTT GCT CCA GGT A	F	Present study

* F, forward; R, reverse.
[1]Primers used only for sequences.
[2]With modifications.

and *Stenus alienus*), and one species that is relatively distant (*Agelosus carinatus*; Caterino et al. 2005; Sikes 2005).

We extracted genomic DNA from samples preserved in 99% EtOH by one of two methods: the conventional phenol–chloroform method or the Chelex DNA preparation protocol of Walsh et al. (1991). We amplified one mitochondrial gene (ribosomal 16S) and three nuclear genes (ribosomal 28S, wingless [Wg], and phosphoenolpyruvate carboxykinase [PepCK]) for each species under standard PCR conditions. The primers are shown in Table 2. Some primers were newly created in this study. PCR products were purified using QIAquick PCR Purification Kits (Qiagen, Tokyo, Japan). Sequencing reactions were performed with a BigDye Terminator Cycle Sequencing Kit (ver. 1.1) following the manufacturer's instructions (Applied Biosystems, Foster City, CA). All samples were run on an ABI377-18 DNA sequencer.

We used Clustal X version 1.83 (Thompson et al. 1997) with default parameter settings for the alignment. The obtained alignments were inspected by eye for obvious misalignments. We did not make a distinction between flight-capable and flightless morphs of *Eusilpha japonica*, a species with dimorphic flight muscles (Ikeda et al. 2007), in the phylogenetic analysis, because their sequences did not differ.

Incongruence among the phylogenetic signals provided by different gene regions was tested with the SH test (Shimodaira and Hasegawa 1999) using a fully optimized model with 1000 bootstrap replicates as implemented in PAUP* version 4.0b10 (Swofford 2002). Maximum-likelihood (ML) topologies of the different genes were compared to one another. An appropriate model for sequence evolution of genes was assessed by hierarchical likelihood-ratio tests (hLRT) using Modeltest version 3.7 (Posada and Crandall 1998).

Bayesian inference (BI) was performed with MrBayes version 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) for the phylogenetic analysis. The models selected by hLRT using MrModeltest version 2 (Nylander et al. 2004) for different genes (16S: GTR + I + G, 28S: GTR + G, Wg: SYM + I + G, PepCK: GTR + I + G) were used in tree searches. Model parameters for each gene were estimated independently using the unlink command. The protein-coding regions (Wg and Pepck) were partitioned by a codon and the model parameters of each codon were estimated independently. The search was run for 1,000,000 generations and sampled every 100 generations. The first 2500 trees were discarded as burnin. In addition, we examined the covarion model, which allows the rate of evolution of a site to vary through time (Tuffley and Steel 1998). Validity of the covarion model compared to the noncovarion model was evaluated by the Bayes factor according to Kass and Raftery (1995); a Bayes factor over 20 indicates strong evidence against the competing hypothesis. We calculated the Bayes factor as the difference between the harmonic means of the covarion and noncovarion models.

ML and equally weighted maximum parsimony (MP) analyses were also conducted in PAUP* version 4.0b10. In the ML analysis, the GTR + I + G model was selected as the appropriate model by hLRT using Modeltest version 3.7. The MP analysis was conducted with heuristic searches. Confidence at each node in both analyses was assessed by 1000 bootstrap replications.

Gaps were treated as a separate presence/absence character using simple indel coding (see Simmons and Ochoterena 2000). These coded gap characters (159 characters) were included in the BI and MP analyses. Coded gap characters were treated as restriction characters (coding = variable) in BI.

For ancestral state reconstructions and comparative analyses, we converted the Bayesian tree, from which outgroup species were pruned, into the ultrametric form using penalized likelihood (Sanderson 2002) as implemented in r8s version 1.7 (Sanderson 2003; divtime method = PL, algorithm = TN). First, cross-validation was performed to select the appropriate smoothing parameter between 1 and 1000 (penalty = add, crossv = yes, cvStart = 0, cvInc = 0.1, cvNum = 31). Second, a penalized-likelihood search was performed using the result of the cross-validation analysis (smoothing = 2).

MEASUREMENT OF ECOLOGICAL TRAITS

We evaluated body length, hindwing length, flight capability, food habit, egg size, and ovariole number of each silphine species. The same beetles were not always measured for all of these characters. Mean relative hindwing length of each species was calculated as the species' mean hindwing length divided by the species' mean body length. We defined flight-capable, flight-dimorphic, and flightless species by whether all, some, or none of the adults of a given species possessed flight muscles (Ikeda et al. 2007).

We used nitrogen stable isotope analysis to determine the food habits of silphine beetles. Methods used for sample preparation and measurement for $\delta^{15}N$ were the same as those described in Ikeda et al. (2007). Data generated in Ikeda et al. (2007) were also included in the present study. The $\delta^{15}N$ ratio can be affected by basal resources in food webs, in addition to primary food resources. For example, the enrichment of ^{15}N through denitrification and wastewater inflow (Mariotti et al. 1982; Wada 1997; Ogawa et al. 2001; Takai 2005) can increase the $\delta^{15}N$ of beetles in the humid soil of riverside and paddy sites. The $\delta^{15}N$ of partially decomposed litter samples collected from the sites (4–6 per site) was significantly higher at riverside sites (1.24 ± 0.84 [SD], $n = 3$) and paddy sites (1.46 ± 2.07, $n = 2$) than at forest sites (-1.96 ± 0.57, $n = 7$; Tukey–Kramer test after significant ANOVA, $P < 0.001$). Therefore, we corrected the $\delta^{15}N$ of samples from riverside and paddy sites using the mean value of forest sites as a standard:

$$\delta^{15}N_{\text{corrected}}\ (‰) = \delta^{15}N_{\text{invertebrates in riverside or paddy sites}} - (\delta^{15}N_{\text{litter in riverside or paddy sites}} - \delta^{15}N_{\text{litter in forest}}).$$

We used the corrected values in the following analysis.

We classified silphine species as "necrophagous" or "predaceous" based on their $\delta^{15}N$. To do this, we used the species-mean $\delta^{15}N$ of four nicrophorine species (*Nicrophorus tenuipes*, *N. maculifrons*, *N. quadripunctatus*, *N. investigator*) that are scavengers that feed only on vertebrate carcasses (Scott 1998; Scott and Gladstein 1993) and six carabid species (*Leptocarabus arboreus*, *L. harmandi*, *Carabus albrechti*, *C. vanvolxemi*, *C. granulatus*, *Cychrus morawitzi*) that are predators that feed on soil invertebrates (e.g., Sota 2000). Their $\delta^{15}N$ was measured by the same method used for silphine beetles. We compared individual $\delta^{15}N$ values for each silphine species to the species' mean values for nicrophorine or carabid species by a one-tailed Dunnett's test using each of the latter groups as a control, after one-way ANOVA comparison of $\delta^{15}N$ in the three groups. We did not distinguish between male and female individuals of each species for the $\delta^{15}N$ because there were no significant differences ($t < 2.3$, $P > 0.09$ for all species). A sequential Bonferroni correction was applied to the significance of the ANOVA for all 16 silphine species. Because the $\delta^{15}N$ of nicrophorine species was higher than that of carabid species (Ikeda et al. 2007), we classified the silphine species as "necrophagous" if the $\delta^{15}N$ of the species was significantly higher than that of carabid species and not significantly different from that of nicrophorine species, and classified them as "predaceous" if the $\delta^{15}N$ of the species was significantly lower than that of nicrophorine species and not significantly different from that of carabid species. There may be predaceous silphine species that are not classified as "predaceous" by this method. *Necrodes surinamensis* has been observed to feed on fly maggots on carcasses (Ratcliffe 1972). However, species that feed mainly

Table 3. Mean (±SD) female body length, hind wing length, flight capability, food habit, egg volume, ovariole number, and egg production of silphine species.

	Female Body length (cm)		Hindwing length (cm)		*Flight capability		Food habit	Egg volume (mm^3)		Ovariole number		Egg production
Oxelytrum discicolle	2.31	(1)	2.38	(1)	+	(1)						
Diamesus osculans	3.95	(1)	2.46±0.245	(5)	+	(5)		0.77	(1)	131.0	(1)	100.9
Necrodes littoralis	2.29±0.197	(4)	2.11±0.185	(8)	+	(8)	necrophagous			88.5±4.95	(2)	
Nd. nigricornis	1.87±0.171	(7)	1.65±0.136	(10)	+	(22)	necrophagous	0.29±0.043	(6)	42.0±1.22	(13)	12.2
Thanatophilus rugosus	1.25±0.057	(8)	1.10±0.063	(14)	+	(19)	necrophagous	0.20±0.016	(6)	24.0±0.00	(8)	4.8
T. sinuatus	1.36±0.347	(7)	1.15±0.056	(13)	+	(16)	necrophagous	0.21±0.039	(5)	24.1±0.35	(8)	5.1
Oiceoptoma nigropunctatum	1.49±0.067	(13)	1.35±0.098	(11)	+	(31)	necrophagous	0.68±0.125	(10)	14.0±0.00	(14)	9.5
Oi. subrufum	1.58±0.086	(10)	1.43±0.123	(14)	+	(20)	necrophagous	0.60±0.149	(6)	14.0±0.00	(8)	8.4
Oi. thoracicum	1.66±0.106	(5)	1.47±0.062	(6)	+	(6)				14.0±0.00	(5)	
Necrophila americana	2.13±0.101	(4)	1.81±0.033	(4)	+	(4)	necrophagous			14.0±0.00	(3)	
Chrysosilpha chloroptera	1.75±0.184	(2)	1.39±0.170	(2)	+	(2)		0.57	(1)	48.0	(1)	27.4
Calosilpha brunnicollis	2.25±0.099	(18)	1.78±0.087	(15)	+	(28)	necrophagous	1.10±0.115	(16)	24.1±0.25	(16)	26.5
Ca. kurosawai	2.11±0.141	(2)	1.86±0.127	(2)	+	(2)				24.0	(1)	
Eusilpha jakowlewi	2.17±0.081	(10)	1.83±0.091	(10)	+	(16)	predaceous	1.58±0.160	(3)	19.3±1.37	(6)	30.6
E. japonica												
(FM present)	2.36±0.076	(11)	1.92±0.130	(17)	+	(111)	predaceous	2.55±0.339	(7)	18.0±1.49	(36)	45.9
(FM absent)	2.45±0.173	(18)	1.94±0.148	(20)	−	(101)	predaceous	2.38±0.287	(9)	17.8±0.73	(33)	42.3
Dendroxena sexcarinata	1.70±0.107	(6)	1.76±0.112	(11)	+	(15)		1.00±0.205	(2)	12.1±0.35	(8)	12.1
Phosphuga atrata	1.62±0.150	(3)	0.91±0.174	(5)	−	(6)	predaceous	1.29	(1)	13.0	(1)	16.8
Silpha tristis	1.66±0.119	(10)	1.38±0.181	(10)	−	(9)		0.85±0.057	(6)	21.5±0.71	(2)	18.3
S. perforata	2.20±0.198	(12)	0.25±0.193	(12)	−	(13)	predaceous	2.62±0.400	(6)	13.8±0.85	(30)	36.1
S. longicornis	1.92±0.124	(9)	0.16±0.051	(16)	−	(16)	predaceous	1.99±0.219	(8)	11.8±1.08	(11)	23.5
S. imitator	2.04±0.097	(4)	0.18±0.038	(4)	−	(4)		2.46±0.480	(2)	12.0±0.00	(2)	29.5

Sample sizes are shown in parentheses.

***+, flight muscle present; −, flight muscle absent; FM, flight muscle.**

on fly maggots feeding on carcasses are not expected to be misclassified as "necrophagous" because the $\delta^{15}N$ is generally 3–4‰ higher in consumers than in their food resource due to ^{15}N enrichment (Minagawa and Wada 1984; Post 2002); thus, such species would have higher $\delta^{15}N$ than nicrophorine species. Because it is impossible or extremely difficult to directly estimate full diet ranges in silphine beetles by, for example, gut content analysis or continuous observation under natural conditions, we believe that our stable isotope analysis is the most appropriate method for the present purposes.

We evaluated the size of eggs produced by a female by average egg volume. We obtained one to five eggs per female by rearing females to oviposition or by dissecting mature females. We estimated egg volume as $\pi LB^2/6$, where L is the major axis and B is the minor axis (e.g., Berrigan 1991). We evaluated mean relative total egg production of females of each species as a product of that species' mean number of ovarioles, which is usually proportional to the number of eggs produced by a female (Price 1975; Fitt 1990; Stewart et al. 1991; R'Kha et al. 1997), and that species' mean individual-average egg volume.

COMPARISON OF REPRODUCTIVE TRAITS BETWEEN FLIGHT-CAPABLE AND FLIGHTLESS SPECIES

To compare female reproductive traits between flight-capable and flightless species while controlling for body size, we conducted an analysis of covariance (ANCOVA) on total egg production, mean egg size, and ovariole number, with mean female body length of the species as a covariate. The flight-dimorphic species *E. japonica* and the extremely large species *Diamesus osculans* (see Table 3) were excluded from the ANCOVA. Mean body

length, total egg production, and mean egg volume were $\log_{10}$-transformed, and mean ovariole number was rank transformed before analysis to improve the heterogeneity of error variances.

ANCESTRAL STATE RECONSTRUCTION AND CORRELATED EVOLUTION

We inferred ancestral states of flight capability and food habit at ancestral nodes by the ML method (Pagel 1994) with a Markov one-parameter model (Lewis 2001) and the MP method using Mesquite version 1.1 (Maddison and Maddison 2006). We set three states for flight capability (flight-capable, flightless, or flight-dimorphic) and two states for food habit (necrophagous or predaceous). *Dendroxena sexcarinata* was pruned in the ancestral state reconstruction of food habit because this species was not classified as necrophagous or predaceous by our methods (see Results).

We tested for correlated evolution between flight capability and food habit using an ML program (Discrete; Pagel 1994). We lumped evolution from the flight-capable state to the flightless or flight-dimorphic states together as loss of flight capability in this analysis. We used a likelihood-ratio test to select the better model between independent (uncorrelated) and dependent (correlated) evolution models.

We used squared-change parsimony to infer ancestral states of continuous characters (mean female body length, relative hindwing length, total egg production, mean egg size, and mean ovariole number) in Mesquite version 1.1. Mean female body length was $\log_{10}$-transformed for the analysis. Total egg production and egg size were divided by (body length)^3 for the analyses to control for body size effects. This method is valid because $\log_{10}$ total egg production and $\log_{10}$ egg size were significantly related to $\log_{10}$ body length, with slopes nearly equal to 3.0 in the extant species, and because we did not find a consistent evolutionary change in body length (see Results). Species lacking some character data were pruned in the analysis of the missing character.

INDEPENDENT CONTRASTS FOR REPRODUCTIVE TRAITS

We examined evolutionary relationships between egg size and number using phylogenetically independent contrasts (Felsenstein 1985) with PDAP module (Midford et al. 2003) in Mesquite version 1.1. The $\log_{10}$-transformed data for female body length, egg size, and ovariole number were used in the analysis. Species for which we could not obtain all character data were pruned in this comparative analysis. Because we did not find significant correlations between the standard deviations of raw contrasts and the absolute values of standardized contrasts for these characters ($r < 0.4$, $n = 15$, $P > 0.1$), we directly used the ultrametric tree (Garland et al. 1992).

Table 4. Numbers of included, variable, and informative sites, and gap characters of individual genes in the dataset.

	Number of sites	Number of variable sites	Number of informative sites	Number of gap characters
16S	566	173	103	38
28S	1005	375	289	117
Wg	500	215	171	3
PepCK	521	188	157	1

To evaluate the effects of evolutionary change in egg number on change in egg size after excluding the effect of evolutionary change in body size, contrasts in egg volume and ovariole number were regressed on those of female body length using a linear function through the origin (Harvey and Pagel 1991), and residual contrasts in egg volume were linearly regressed through the origin on those of ovariole number.

Results

PHYLOGENETIC ANALYSIS

A total of 2592 aligned variable nucleotide sites and 159 gap characters were used for the phylogenetic analysis (Table 4). The likelihood of the topology of the Wg gene tree was significantly lower than those of the other gene topologies (SH test: $P < 0.01$) because we could not determine the sequence of the Wg gene in one outgroup species, *A. carinatus*. When we conducted the SH test excluding *A. carinatus* from all genes, there were no significant differences among genes. Accordingly, we conducted the phylogenetic analysis using all genes simultaneously.

Because the covarion model was more valid than the noncovarion model in BI (Bayes factor = 65.0), we used the Bayesian tree constructed from the model including the covarion. The topologies constructed by the Bayesian, ML, and MP analyses yielded highly congruent results (Fig. 1). The inclusion of gap characters improved bootstrap values of the MP tree, but had little effect on posterior probabilities of the BI tree. All analyses identified the monophyly of the Silphidae and provided strong support for the monophyly of the Silphinae, Nicrophorinae, and all genera examined. The tree of the Silphinae constructed in this study was basically congruent with those shown by Dobler and Müller (2000) and Sikes et al. (2005) except for the positions of the *Necrodes* + *Diamesus* group and the *Thanatophilus* group. *Ptomascopus* was the most basal group in the Nicrophorinae as shown by Dobler and Müller (2000). Some of the species groups within *Nicrophorus* noted by Peck and Anderson (1985) and Sikes et al. (2006) were supported (investigator group of *N. investigator* and *N. tomentosus*; nepalensis group of *N. maculifrons* and *N. quadripunctatus*).

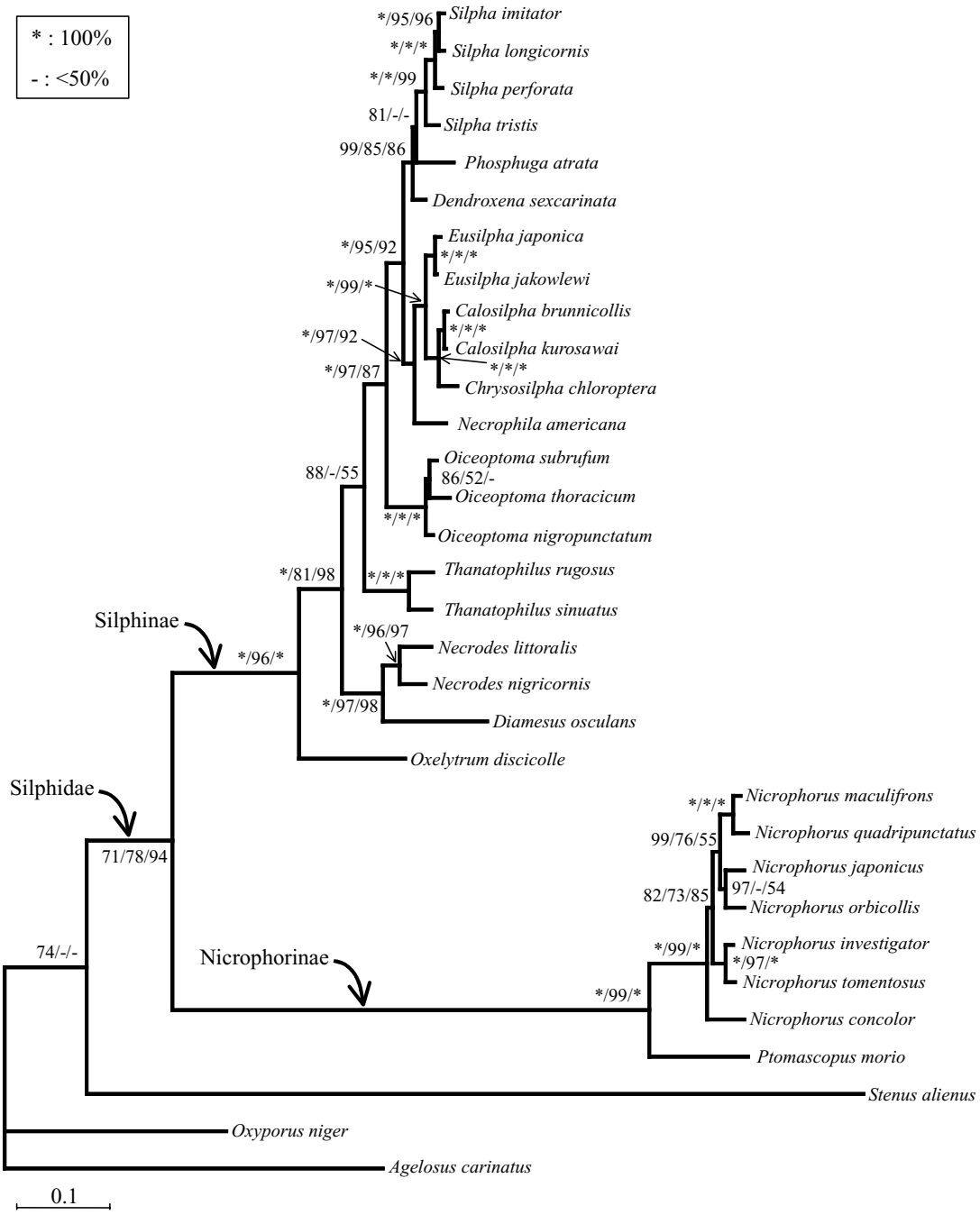

Figure 1. **Phylogenetic relationships of 29 silphid species (21 silphine species and eight nicrophorine species) and three staphylinid species from a Bayesian analysis based on a combined analysis of nuclear and mitochondrial sequences. The figures or symbols at nodes represent Bayesian posterior probability/ML bootstrap percentage/MP bootstrap percentage. The scale bar depicts the expected number of substitutions per site.**

COMPARISON OF REPRODUCTIVE TRAITS BETWEEN FLIGHT-CAPABLE AND FLIGHTLESS SPECIES

The ecological characters of each silphine species are shown in Table 3. Fifteen silphine species were regarded as flight-capable and five species were regarded as flightless. Almost equal numbers of individuals with and without flight muscles were observed in *E. japonica.* Most flightless species had shorter hindwings than flight-capable species.

The δ^{15}N of nicrophorine species (7.58 $\pm$ 0.46 [SD]) was significantly higher than that of carabid species (2.77 $\pm$ 0.60; $t_8 = 13.5$, $P < 0.001$). Eight silphine species had mean δ^{15}N ranging from 5.8 to 10.1‰ and were classified as necrophagous

Table 5. δ^{15}N (mean±SD) of silphine species with numbers of individuals examined and their collection localities, and results of food habit classification of the silphine species (necrophagous or predaceous).

				Classification into food habit group		
				P values of Dunnett tests		
	δ^{15}N	*n*	Number of localities	Nicrophorine control	Carabid control	Food habit
Necrodes littoralis	6.6±1.97	2	2	1.00	**0.05**	necrophagous
Nd. nigricornis	10.1±1.42	7	2	0.14	**0.00**	necrophagous
Thanatophilus rugosus	8.1±2.04	8	2	1.00	**0.00**	necrophagous
T. sinuatus	7.5±1.91	9	3	1.00	**0.00**	necrophagous
Oiceoptoma nigropunctatum	6.3±1.46	19	6	0.66	**0.00**	necrophagous
O. subrufum	8.3±2.29	19	7	0.99	**0.00**	necrophagous
Necrophila americana	5.8±1.45	6	1	0.47	**0.03**	necrophagous
Calosilpha brunnicollis	8.9±1.96	17	6	0.71	**0.00**	necrophagous
Eusilpha jakowlewi	4.2±1.25	8	3	**0.05**	0.18	predaceous
E. japonica						
(FM present)	2.6±1.87	40	11	**0.00**	1.00	predaceous
(FM absent)	2.1±1.28	35	10	**0.00**	0.99	predaceous
Dendroxena sexcarinata	5.4±0.21	2	2	0.61	0.36	
Phosphuga atrata	3.8±1.29	3	3	**0.03**	0.99	predaceous
Silpha perforata	3.8±1.39	11	8	**0.00**	0.87	predaceous
S. longicornis	2.8±1.03	8	4	**0.00**	1.00	predaceous

See text for detailed methods of this classification. Significant values (*P* <0.05) are shown in bold.

*** FM, flight muscle.**

(Tables 3 and 5). Five silphine species had mean δ^{15}N ranging from 2.1 to 4.2‰ and were classified as predaceous.

Mean body length did not differ significantly between flight-capable and flightless species (Table 3; one-way ANOVA: $F_{1,12} = 1.18$, $P = 0.30$). We conducted all ANCOVAs without the interaction term between body length and flight capability, which was not significant ($P > 0.1$). Flightless species had 1.4 times higher total egg production than flight-capable species (flight capability: $F_{1,11} = 4.73$, $P = 0.052$; body size: $F_{1,11} = 53.65$, $P < 0.001$; Fig. 2A). Eggs of flightless species were two times larger than those of flight-capable species (flight capability: $F_{1,11} = 10.22$, $P = 0.008$; body size: $F_{1,11} = 17.46$, $P = 0.002$; Fig. 2B). Flight-capable species had 1.7 times more ovarioles than flightless species (flight capability: $F_{1,11} = 5.53$, $P = 0.038$; body size: $F_{1,11} = 0.01$, $P = 0.934$; Fig. 2C).

No ecological characters except flight capability were significantly different between flight-capable and flightless morphs in *E. japonica* (female body length: $t_{27} = 1.57$, $P = 0.13$; relative hindwing length: $t_{35} = -0.49$, $P = 0.63$; total egg production: $t_9 = -1.23$, $P = 0.25$; egg volume: $t_{14} = -1.06$, $P = 0.31$, ovariole number: $t_{67} = 0.85$, $P = 0.40$; Table 3 and 5). Accordingly, we used the mean values of all individuals as character data for this species. Reproductive traits of the flight dimorphic species *E. japonica* appeared to be intermediate between those of flight-capable and flightless species (Fig. 2A–C).

ANCESTRAL STATE RECONSTRUCTION AND INDEPENDENT CONTRASTS

Ancestral state reconstructions of flight capability in the ML and MP analyses were congruent (Fig. 3A). The ML analysis supported one of the three character states (flight-capable, flightless, or flight-dimorphic) at a probability of more than 90% for all nodes (Fig. 3A). Flight muscles were lost twice in separate lineages, including one complete degeneration and one evolution to dimorphism. The ML analysis supported a necrophagous lifestyle at high probability in the basal nodes, and the MP analysis implied that the evolutionary change from a necrophagous to a predatory lifestyle occurred twice (Fig. 3B), suggesting that predaceous species were derived from necrophagous species.

The predaceous state was supported at the node before the evolution to dimorphism, suggesting that the evolution of flight dimorphism occurred after a food habit change from necrophagous to predaceous. However, we could not determine with certainty whether the food habit change preceded the complete degeneration event. Comparison of dependent and independent evolution models significantly supported correlated evolution between flight capability and food habit (likelihood ratio [LR] = 10.65, $P < 0.05$).

Female body length did not change consistently in the lineage leading to flightless or flight-dimorphic species. Relative hindwing length decreased after the complete degeneration of flight

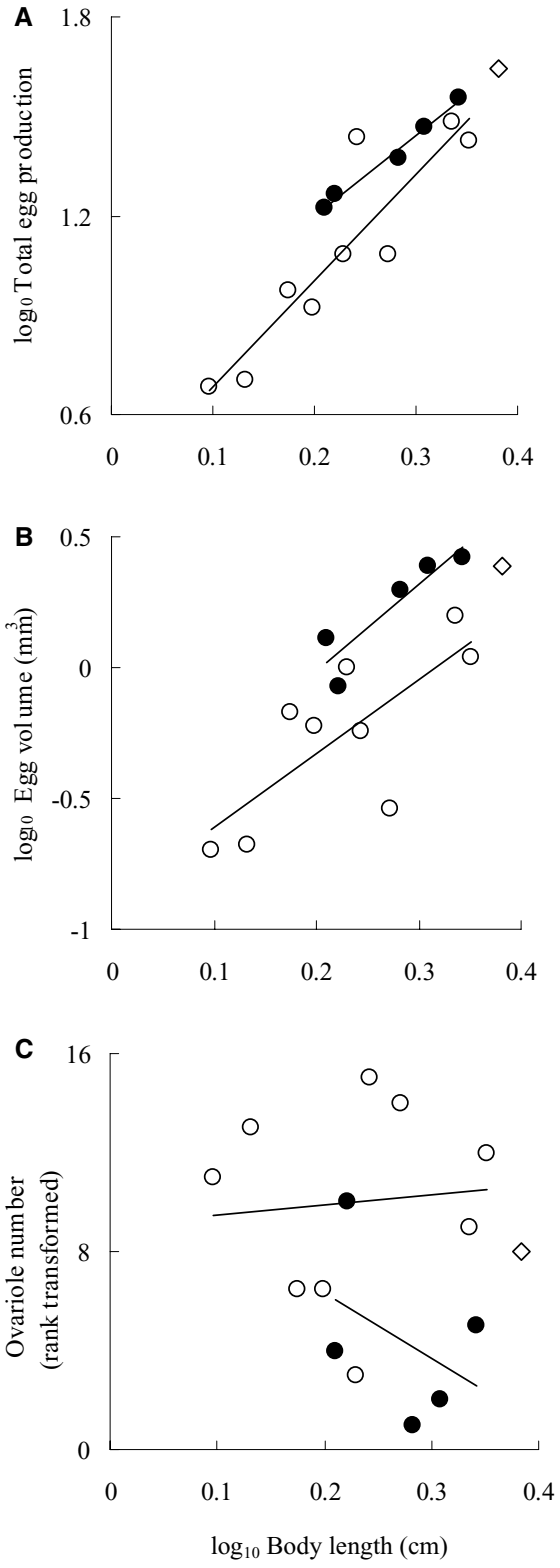

Figure 2. **Comparisons of (A) total egg production, (B) egg volume, and (C) ovariole number between flight-capable (○) and flightless (•) species of Silphinae. A linear regression is shown for each flight-capable and flightless species. The flight-dimorphic species *E. japonica* is shown with white diamonds.**

muscles (Fig. 4A), supporting Roff's (1986) prediction of the evolutionary sequence. Total egg production increased following the complete degeneration of flight muscles (Fig. 4B). Egg volume increased following the food habit change from necrophagous to predaceous (Fig. 4C). Ovariole number decreased following the food habit change in the lineage leading to predaceous species (Fig. 4D). Residual contrasts from the regression of egg volume on body length were negatively and highly correlated with those from the regression of ovariole number on body length ($r = 0.79$, $P < 0.001$; Fig. 5), suggesting that the evolution of increased egg size is associated with the evolution of decreased egg number in the Silphinae.

Discussion

The tree constructed in this study strongly supports the monophyly of the Silphinae and was basically congruent with previous tree (Dobler and Müller 2000), providing a reliable basis for our evolutionary analysis of ecological traits. Although some differences exist between our results and previous results, we believe that our results are more reliable because we used a larger number of genes with appropriate evolutionary rates.

The ancestral state reconstruction using our robust Silphidae tree suggests that the ancestral food habit in the Silphinae was necrophagy. Given that nicrophorine species are necrophagous (Scott 1998; Scott and Gladstein 1993), Silphinae and Nicrophorinae should be derived from a necrophagous ancestor. This necrophagous ancestor might have been derived from an ancestral species with a saprophagous lifestyle, because in Staphylinidae there are some species that feed on both live and dead invertebrates (e.g., Leschen 1993). A phylogenetic analysis including the staphylinid species closest to the Silphidae as outgroup species and ancestral state reconstruction including them may be necessary to address this issue.

The ancestral state reconstructions of flight capability implied that loss of flight muscles occurred twice in the Silphinae (one complete degeneration event and one evolution to flight dimorphism). This result and the ancestral state reconstruction of food habit suggest that predaceous species were derived from a necrophagous ancestor and that the evolution of flight muscle dimorphism occurred after the evolutionary change from a necrophagous to a predatory lifestyle. Furthermore, the correlated evolution of flight capability and food habit was supported. These findings are consistent with our prediction that an evolutionary change from a necrophagous to a predatory lifestyle resulted in the loss of flight in the Silphinae. It is generally presumed that flightlessness evolved in insects in response to changes in the habitat environment or degree of habitat isolation (Harrison 1980; Roff 1990; Wagner and Liebherr 1992). Our results suggest that

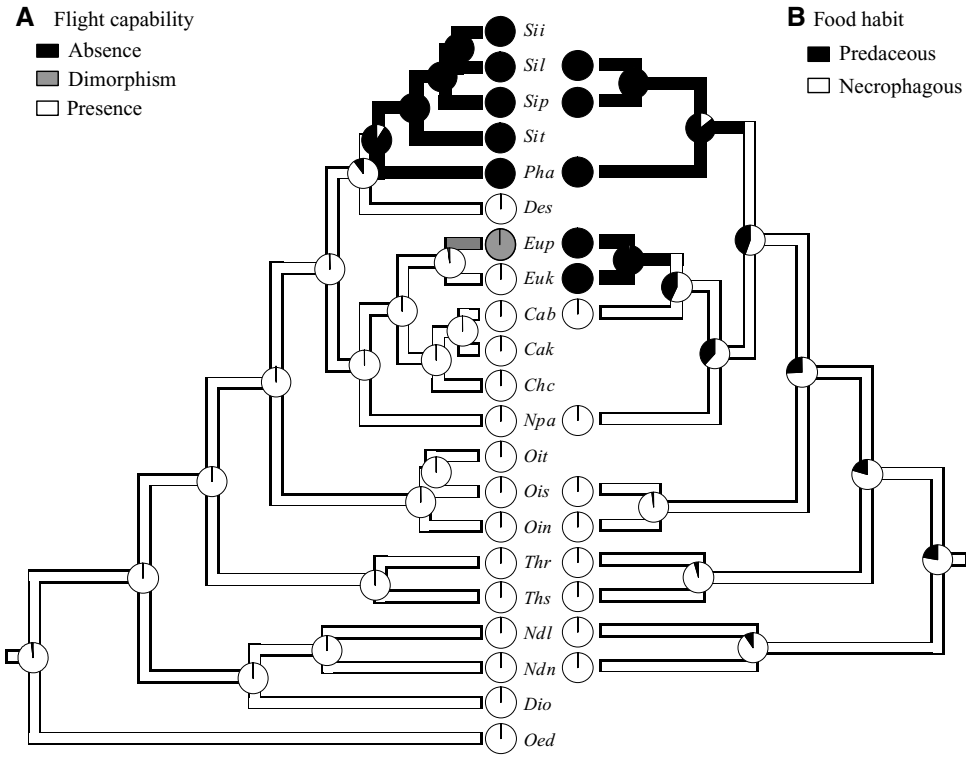

Figure 3. ML and MP reconstructions of (A) flight capability and (B) food habit in the Silphinae using Mesquite version 1.1 (Maddison and Maddison 2006). The pie graph indicates the ML support for the ancestral state at each node. The branch pattern indicates the MP reconstruction. Abbreviations for species names are as shown in Table 1.

species' own evolutionary change could cause the loss of flight, independently of changes in the environmental circumstances per se.

We did not find any silphine species that had significantly higher δ^{15}N than nicrophorine species, suggesting that no species examined in this study mainly feed on fly maggots on vertebrate carcasses. There might be some species that are primarily necrophagous but feed on the fly maggots opportunistically. *Necrodes nigricornis* had a relatively high δ^{15}N (2.5‰ higher than the average δ^{15}N of nicrophorine species; Table 5). This value is similar to that of *Ontholestes gracilis* (9.5 ± 1.76, $n = 6$; H. Ikeda unpubl. data), a staphylinid that feeds on not only carcasses but also on flies and the fly maggots on carcasses (Hirano 1982, H. Ikeda pers. obs.). Considering that *Necrodes* species are sister to a large group of other Silphinae that show derived character states, some ancestors of the Silphinae and Silphidae might feed on the fly maggots as well as vertebrate carcasses.

Our diet estimation using the nitrogen stable isotope ratio has a limited ability to identify predaceous species depending on other than soil invertebrates. It may not be able to distinguish necrophagous species from those that feed exclusively on fly maggots if they forage on vertebrate dung as well as carcasses. In addition, we could not determine the feeding habit of *D. sexcarinata* using the nitrogen stable isotope ratio, although adults of this species were observed feeding on lepidopteran larvae in an arboreal habitat (Sugiura and Yamazaki 2007). Our breeding experiments and field observations (H. Ikeda unpubl. data) help shed light on these questions. Most species that we classified as necrophagous, including *Necrodes* species, developed from larvae to adults and reached sexual maturity when fed rotten beef only; we never observed them feeding on the abundant fly maggots on the rotten chicken in bait traps. Therefore, none of the silphine species that were examined would feed mainly on fly maggots. The larvae of *D. sexcarinata* could develop to the adult stage when fed either rotten beef or invertebrates only, and we often found adults and larvae of this species on rotten chicken in bait traps. Thus, *D. sexcarinata* probably feeds on both vertebrate carcasses and invertebrates in nature.

Flightless species had 1.4 times higher total egg production than flight-capable species. The ancestral state reconstruction suggests that allocation to total egg production increased with the complete flight muscle degeneration event. These findings are consistent with our prediction that flight loss resulted in selection for increased relative reproductive investment in the Silphinae. High total egg production, similar to that in flightless species and nodes, did not occur in any other lineages except that leading to *Chrysosilpha chloroptera*, whose ecology is poorly known. Thus, the evolution of flight loss was responsible for

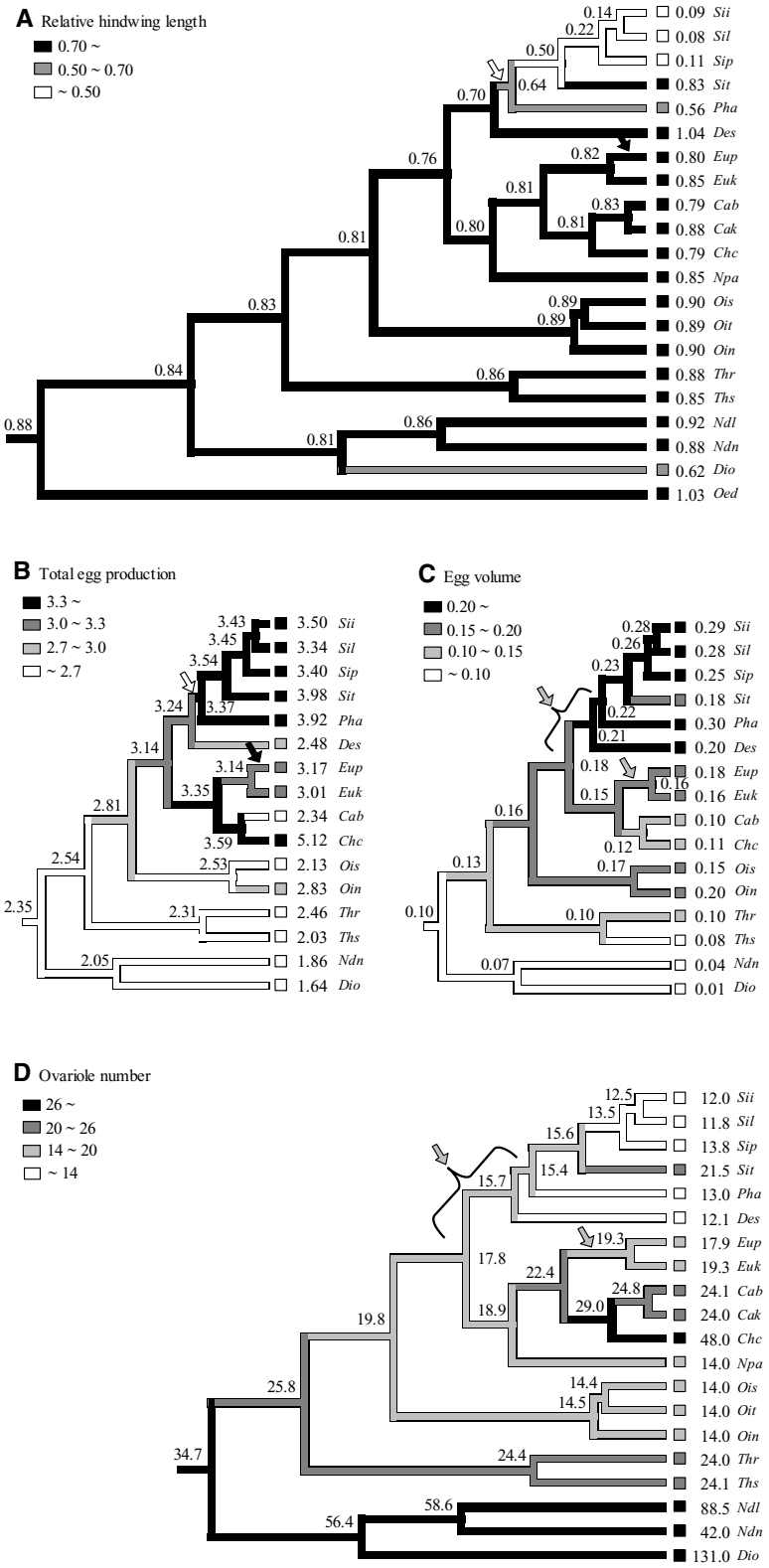

Figure 4. Squared-change parsimony reconstructions of (A) relative hindwing length (hindwing length/body length), (B) relative total egg production, (C) egg volume, and (D) ovariole number in Silphinae using Mesquite version 1.1 (Maddison and Maddison 2006). Relative total egg production and egg volume were divided by (body length)^3. Trait values for each node and species are shown. In (B), an open arrow indicates the branch where flight muscles were completely lost and a solid arrow indicates the branch where a change to flight muscle dimorphism occurred. Gray arrows in (C) and (D) represent the branches where a food habit change was inferred in the MP analysis. Abbreviations for species names are as shown in Table 1.

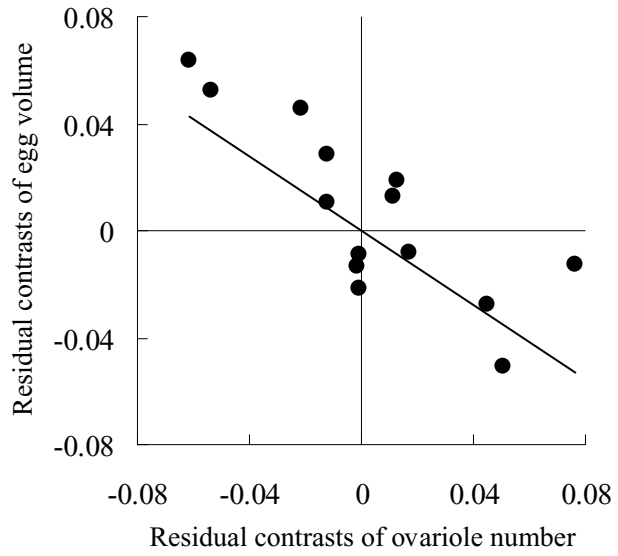

Figure 5. Relationship between residual contrasts of ovariole number and egg volume from regressions on body length. A linear regression line through the origin is shown.

interspecific variation in total egg production among the extant silphine species.

We focused on flight muscle degeneration events to examine the correlated evolution of flight loss and the increase in total egg production, but other processes of flight loss might have influenced the evolutionary process of reproductive allocation. Ikeda et al. (2007) noted that flight activity varies among some flight-capable silphine species. Thus, a trade-off might have existed between total egg production and flight activity before flight muscle degeneration. To fully elucidate the correlated evolution of flight loss and reproductive allocation, the evolution of flight characters such as flight frequency, duration of each flight, and flight muscle mass should also be examined.

Eggs of flightless species were twice as large as those of flight-capable species. The ancestral state reconstruction suggests that egg size increased following the food habit change from necrophagous to predaceous. These findings are consistent with our prediction that evolution of a predatory lifestyle resulted in selection for increased egg size in the Silphinae. The ancestral state reconstruction also suggests that egg number decreased following the food habit change, and the independent contrast analysis suggests a negative correlation between egg size and number. Therefore, the evolution of increased egg size was caused by the trade-off between egg size and number. These evolutionary changes were responsible for interspecific variation in egg size and number among the extant species.

The life-history pattern in extant species of Silphinae was shaped by a sequence of coadaptation processes. Although more advanced adaptations using vertebrate carcasses, i.e., parental care on carcasses, occur in the Nicrophorinae, egg size increased with a decrease in egg number related to a food habit change from necrophagous to predaceous in the Silphinae. This food habit change induced ancestral silphine beetles to lose their flight capability and increase their reproductive allocation. Life-history evolution is a complex process involving various traits and, thus, the coadaptation process related to flight loss varies widely among insect taxa. Intriguing evolutionary processes related to flight loss might be independent of changes in habitat environment or isolation that was invoked in other insect taxa.

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LITERATURE CITED

Balduf, W. V. 1935. The bionomics of entomophagous coleoptera. John S. Swift, New York.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. Am. Zool. 36:216–236.

Berrigan, D. 1991. The allometry of egg size and number in insects. Oikos 60:313–321.

Caterino, M. S., T. Hunt, and A. P. Vogler. 2005. On the constitution and phylogeny of Staphyliniformia (Insecta:Coleoptera). Mol. Phylogenet. Evol. 34:655–672.

Dobler, S., and J. K. Müller. 2000. Resolving phylogeny at the family level by mitochondrial cytochrome oxidase sequences: phylogeny of carrion beetles (Coleoptera, Silphidae). Mol. Phylogenet. Evol. 15:390–402.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Fitt, G. P. 1990. Variation in ovariole number and egg size of species of *Dacus* (Diptera; Tephritidae) and their relation to host specialization. Ecol. Entomol. 15:255–264.

Fox, C. W. 1994. The influence of egg size on offspring performance in the seed beetle, *Callosobruchus maculatus*. Oikos 71:321–325.

Fox, C. W., and M. E. Czesak. 2000. Evolutionary ecology of progeny size in arthropods. Annu. Rev. Entomol. 45:341–369.

Garland, T. J., P. H. Harvey, and A. R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Syst. Biol. 41:18–32.

Hanski, I., M. Saastamoinen, and O. Ovaskainen. 2006. Dispersal-related life-history trade-offs in a butterfly metapopulation. J. Anim. Ecol. 75:91–100.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annu. Rev. Ecol. Syst. 11:95–118.

Harusawa, K. 1996. Rearing of the Japanese silphid *Calosilpha brunneicollis*. Nat. Ins. 31:13–17.

Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford Univ. Press, Oxford.

Hirano, Y. 1982. *Ontholestes gracilis* preys on one of the species of Lucilini. Kanagwa-Chuho 67:23.

Hosoya, T., and K. Araya. 2005. Phylogeny of Japanese stag beetles (Coleoptera:Lucanidae) inferred from 16S mtrRNA gene sequences, with reference to the evolution of sexual dimorphism of mandibles. Zool. Sci. 22:1305–1318.

Hosoya, T., M. Honda, and K. Araya. 2001. Genetic variations of 16S rRNA gene observed in *Ceruchus lignarius* and *Dorcus rectus rectus* (Coleoptera:Lucanidae). Entomol. Sci. 4:335–344.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Ikeda, H., K. Kubota, T. Kagaya, and T. Abe. 2007. Flight capabilities and feeding habits of silphine beetles: are flightless species really "carrion beetles"? Ecol. Res. 22:237–241.

James, J. W. 1974. Genetic covariances under the partition of resources model. Appendix 1 in Sheridan and Barker (1974). Aust. J. Biol. Sci. 27:99–101.

Jonsson, M. 2003. Colonisation ability of the threatened tenebrionid beetle *Oplocephala haemorrhoidalis* and its common relative *Bolitophagus reticulatus*. Ecol. Entomol. 28:159–167.

Kass, R. E., and A. E. Raftery. 1995. Bayes factors. J. Am. Stat. Assoc. 90:773–795.

Kurosa, K. 1995. Breeding and observation of ground beetles. Pp. 136–149 *in* J. Aoki, and H. Watanabe, eds. Life in the soil, Tsukiji-Syokan, Tokyo.

Leschen, R. A. B. 1993. Evolutionary patterns of feeding in selected Staphylinoidea (Coleoptera): shifts among food textures. Pp. 59–104 *in* C. W. Schaefer, and R. A. B. Leschen, eds., Functional morphology of insect feeding, Entomological Society of America, MD.

Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50:913–925.

Lovei, G. L., and K. D. Sunderland. 1996. Ecology and behavior of ground beetles (Coleoptera:Carabidae). Annu. Rev. Entomol. 41:231–256.

Loxdale, H. D., and G. Lushai. 1998. Molecular markers in entomology. Bull. Entomol. Res. 88:577–600.

Maddison, W. P., and D. R. Maddison. 2006. Mesquite: a modular system for evolutionary analysis. Version 1.12. (http://mesquiteproject.org).

Mariotti, A., J. C. Germon, and A. Leclerc. 1982. Nitrogen isotope fractionation associated with the $NO_2 \rightarrow N_2O$ step of denitrification in soils. Can. J. Soil Sci. 62:227–241.

Marshall, D. J., C. N. Cook, and R. B. Emlet. 2006. Offspring size effects mediate competitive interactions in a colonial marine invertebrate. Ecology 87:214–225.

Midford, P. E., T. Garland, Jr., and W. P. Maddison. 2003. PDAP Package.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta 48:1135–1140.

Montague, J. R., R. L. Mangan, and W. T. Starmer. 1981. Reproductive allocation in the Hawaiian Drosophilidae: egg size and number. Am. Nat. 118:865–871.

Nylander, J. A. A., F. Ronquist, J. P. Huelsenbeck, and J. L. Nieves-Aldrey. 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53:47–67.

Ogawa, N. O., T. Koitabashi, H. Oda, T. Nakamura, N. Ohkouchi, and E. Wada. 2001. Fluctuations of nitrogen isotope ratio of gobiid fish (Isaza) specimens and sediments in Lake Biwa, Japan, during the 20th century. Limnol. Oceanogr. 46:1228–1236.

Pagel, M. 1994. Detecting correlated evolution on phylogenies, a general method for the comparative analysis of discrete characters. Proc. R. Soc. Lond. B 255:37–45.

Parker, G. A., and M. Begon. 1986. Optimal egg size and clutch size: effects of environment and maternal phenotype. Am. Nat. 128:573–592.

Peck, S. B., and R. S. Anderson. 1985. Taxonomy, phylogeny and biogeography of the carrion beetles of Latin America (Coleoptera:Silphidae). Quaest. Entomol. 21:247–317.

Posada, D., and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Price, P. W. 1975. Reproductive strategies of parasitoids. Pp. 87–111 *in* Price, P. W. ed. Evolutionary strategies of parasitic insects and mites, Plenum Pub. Corp., New York.

Ratcliffe, B. C. 1972. The natural history of *Necrodes surinamensis* (Fabr.) (Coleoptera: Silphidae). Trans. Am. Ent. Soc. 98:359–410.

R'Kha, S., B. Moreteau, J. A. Coyne, and J. R. David. 1997. Evolution of a lesser fitness trait: egg production in the specialist *Drosophila sechellia*. Genet. Res. 69:17–23.

Roff, D. A. 1986. The evolution of wing dimorphism in insects. Evolution 40:1009–1020.

——. 1990. The evolution of flightlessness in insects. Ecol. Monogr. 60:389–421.

——. 1991. Life history consequences of bioenergetic and biomechanical constraints on migration. Am. Zool. 31:205–215.

——. 1994. The evolution of flightlessness: is history important? Evol. Ecol. 8:639–657.

——. 2002. Life history evolution. Sinauer Associates Inc, MA.

Roff, D. A., and D. J. Fairbairn. 1991. Wing dimorphisms and the evolution of migratory polymorphisms among the Insecta. Am. Zool. 31:243–251.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19:101–109.

——. 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19:301–302.

Scott, M. P. 1998. The ecology and behavior of burying beetles. Annu. Rev. Entomol. 43:595–618.

Scott, M. P., and D. S. Gladstein. 1993. Calculating males? An empirical and theoretical examination of the duration of parental care in burying beetles. Evol. Ecol. 7:362–378.

Shimodaira, H., and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16:1114–1116.

Sikes, D. S. 2005. Silphidae Latreille, 1807. Pp. 288–296 *in* R. G. Beutel, and R. A. B. Leschen, eds., Coleoptera, Beetles: morphology and systematics (Archostemata, Adephaga, Myxophaga, Polyphaga Partim). Walter De Gruyter Inc, Berlin.

Sikes, D. S., S. T. Trumbo, and S. B. Peck. 2005. Silphinae. Version 07 February 2005(under construction). (http://tolweb.org/ Silphinae/ 26994/2005.02.07) in The Tree of Life Web Project, (http://tolweb.org/).

Sikes, D. S., R. B. Madge, and S. T. Trumbo. 2006. Revision of *Nicrophorus* in part: new species and inferred phylogeny of the nepalensis-group based on evidence from morphology and mitochondrial DNA (Coleoptera:Silphidae:Nicrophorinae). Invertebr. Syst. 20: 305–365.

Simmons, M. P., and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49:369–381.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annu. Entomol. Soc. Am. 87:651–701.

Smith, C. C., and S. D. Fretwell. 1974. The optimal balance between size and number of offspring. Am. Nat. 108:499–506.

Sota, T. 2000. Carabus in four seasons: life history evolution and species diversity. Kyoto Univ. Academic Press, Kyoto. (in Japanese).

Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press. Oxford.

Stewart, L. A., J.-L. Hemptinne, and A. F. G. Dixon. 1991. Reproductive tactics of ladybird beetles: relationships between egg size, ovariole number and developmental time. Funct. Ecol. 5:380–385.

Sugiura, S., and K. Yamazaki. 2007. Outbreak of *Ivela auripes* (Lepidoptera, Lymantridae) and its arboreal predator *Dendroxena sexcarinata* (Coleoptera, Silphidae). Trans. Lepid. Soc. Japan 58:40–42.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b. Sinauer Associates, Sunderland, MA.

Takai, N. 2005. Carbon and nitrogen stable isotope ratios indicative of organic matter flow in the ecosystem of the Seto Inland Sea, Japan. Jap. J. Ecol. 55:269–285.

Tanaka, S. 1993. Allocation of resources to egg production and flight muscle development in a wing dimorphic cricket, *Modicogryllus confirmatus*. J. Ins. Phys. 39:493–498.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 24:4876–4882.

Tuffley, C., and M. Steel. 1998. Modeling the covarion hypothesis of nucleotide substitution. Math. Bios. 147:63–91.

Van Noordwijk, A. J., and G. de Jong 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. Am. Nat. 128:137–142.

Wada, E. 1997. View of the stable isotope analysis in ecology. Kaiyo Monthly 7:436–442 (in Japanese).

Wagner, D. L., and J. K. Liebherr. 1992. Flightlessness in insects. Trends Ecol. Evol. 7:216–220.

Wallin, H., P. A. Chiverton, B. S. Ekbom, and A. Borg. 1992. Diet, fecundity and egg size in some polyphagous predatory carabid beetles. Entomol. Exp. Appl. 65:129–140.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513.

Whiting, M. F. 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. Zool. Scr. 31:93–104.

Winkler, D. W., and K. Wallin. 1987. Offspring size and number: a life history model linking effort per offspring and total effort. Am. Nat. 129:708–720.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annu. Rev. Entomol. 42:207–231.

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