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Morphology and evolution of the cynipoid egg (Hymenoptera)

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We describe gross egg morphology and provide the first data on eggshell ultrastructure in cynipoids (Hymenoptera) based on species representing three distinctly different life histories: internal parasitoids of endopterygote larvae, gall inducers and phytophagous inquilines (guests in galls). We then use existing phylogenetic hypotheses to identify putative changes in egg structure associated with evolutionary life-history transitions. We find four major structural changes associated with the shift from parasitoids laying their eggs inside a host larva to gall inducers laying their eggs in or on plants: (1) from a narrow and gradually tapering gross form to a distinct division into a stout body and a long and thin stalk; (2) from a thin to a thick eggshell; (3) from a flexible to a rigid endochorion; and (4) from crystal bundles with shifting orientation in the exochorion to layers of parallel crystal rods. By contrast, we find no major changes in egg structure associated with the transition from gall inducers to inquilines. Comparison between pre- and post-oviposition eggs of one gall inducer and one inquiline suggests that mechanical stress during the passage through the egg canal gives rise to numerous tiny stress fractures in the boundary separating the exo- and endochorion. In one of the gall inducers, *Diplolepis rosae*, that end of the egg, which is inserted into the plant, has a specialized and apparently porous shell that may permit chemical exchange between the embryo and the plant. Other structures that could facilitate chemical communication with the host plant through the eggshell were, however, not observed in the eggs of gall inhabitants. *Zoological Journal of the Linnean Society*, 2003, **139**, 247–260.

ADDITIONAL KEYWORDS: chorion – eggshell – gall inducer – parasitoid – SEM – TEM – ultrastructure.

INTRODUCTION

The parasitic-wasp superfamily Cynipoidea includes insects with three distinctly different life modes (Ronquist, 1999). The parasitoids, represented by the families Austrocynipidae, Ibaliidae, Liopteridae and Figitidae, oviposit into embryos or larvae of endopterygote insects, inside which their larvae develop. The gall inducers, represented by the family Cynipidae, oviposit into plant tissue (e.g. buds). The egg is often positioned on the plant surface with only the tip inserted into the epidermis but sometimes the whole egg is embedded in plant tissue. The attacked plant tissue eventually produces a gall, inside which the wasp larva feeds on a specially developed, nutritious layer of plant cells lining its chamber. The inquilines, also belonging to the Cynipidae, having apparently lost the ability to initiate galls, still retain the ability to modify plant development. The inquiline female deposits her eggs in young galls induced by other cynipids, sometimes killing the original gall-inducing larva by stabbing it to death with her ovipositor (Shorthouse, 1980). New larval chambers later develop around the inquiline larvae and provide them with food.

The last decade has seen swift progress in the understanding of cynipoid relationships (Ronquist, 1994, 1995, 1999). Phylogenetic evidence indicates that cynipoids were originally insect parasitoids,

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like most of their relatives among the parasitic Hymenoptera. The gall inducers evolved from parasitoids developing on hosts inside plants, and the gall inducers later gave rise to the inquilines.

Although some of the most complex insect galls known are induced by cynipids, the mechanisms used by them to manipulate plant development are still unknown. It is commonly assumed that chemical signals produced by the young larva are primarily responsible (Rohfritsch, 1992) but chemicals injected into the plant by the ovipositing female, or secreted by the embryo and passed through the eggshell, have also been implied (Magnus, 1914). An entirely different hypothesis holds that cynipid gall induction is caused by, or at least facilitated by, symbiotic viruses, presumably injected into the plant by the ovipositing female (Cornell, 1983). Virus-like particles suppressing the cellular immunity of the host are known from derived members of the insect-parasitic sister-group of the gall wasps, the Figitidae (Rizki & Rizki, 1990).

The phylogenetic data available on cynipoids allow identification of anatomical changes associated with the origin of gall inducers and inquilines. These changes may give important clues to the likely gall-induction mechanisms. One of the aims of the present study is to apply such a phylogenetic approach to elucidate the role of the cynipid egg in gall induction.

Little is known about cynipoid eggs but the gross morphology has been described for some species. The egg consists of an egg body and a projection or peduncle (pedicel) at the anterior end (Fig. 1). During oviposition, the egg body, i.e. the posterior end of the egg, passes through the narrow ovipositor canal first, followed by the peduncle. When the egg body is pressed through the canal, the egg content flows into the peduncle (Frühauf, 1924; Bronner, 1985). Once the egg body protrudes at the end of the ovipositor, the egg content flows back into it. Thus, the peduncle appears mainly to be a device for facilitating the passage of a large egg through a narrow egg canal. The peduncle has been seen to degenerate in *Trybliographa* (formerly *Eucoila*) *keilini* Kieffer and *Kleidotoma japonica* Huzimatu (Figitidae: Eucoilinae) after oviposition (Keilin & Pluvinel, 1913; Huzimatu, 1940).

In parasitic wasps, the micropyle is normally at the anterior end of the egg. It may be exposed as in *Nasonia vitripennis* (Walker) (Chalcidoidea: Pteromalidae) (King, 1962) or situated inside a fold of the chorion, visible as a slight bulge on the surface of the egg body as in *Venturia* sp. (Ichneumonidae) (Rotheram, 1972). In accordance with this, the micropyle has been reported to be either at the apex of the peduncle (Wishart & Monteith, 1954) or slightly more basally on the peduncle, close to the egg body (Leuckart, 1855) in cynipoids. In the oak gall wasps (Cynipini), the embryo initially develops with its head directed towards the anterior end of the egg (Magnus, 1914). Later, it turns around and completes its development with the head facing the posterior end, through which it eventually emerges (Magnus, 1914; Ovruski, 1994). The development is likely to be similar in other cynipoids.

There are no published studies of the ultrastructure of the cynipoid eggshell but there are some studies of eggs of other parasitic Hymenoptera. The insect eggshell is normally described as consisting of two main layers: the inner vitelline envelope (VE) and the outer chorion, the latter of which is usually further divided into an endochorion (EN) and an exochorion (EX) (Margaritis, 1985). The egg of the parasitoid *Venturia* sp. (Ichneumonidae) is deposited inside the host, and has a relatively thin VE, supposedly in order to allow uptake of nutrients from the host's body fluids (Rotheram, 1972; as *Nemeritis*). The EN is 0.8–0.9 μm thick, and contains crystalline elements. Furneaux & Mackay (1972) and Sahlén (1994a) describe similar crystalline arrangements in the chorion of several other groups of insects. The major component of the crystalline element is a protein, the amino acid composition of which appears to vary between different insect groups (Furneaux & Mackay, 1972). The thickness of the EX varies enormously between different parts of the *Venturia* egg (3–30 μm). It consists of long projections and dense material filling the space between the projections. The eggshell of the endoparasitoid *Cardiochiles nigriceps* (Viereck) (Braconidae) is similar to that of *Venturia,* in having an inner crystalline layer and microvillous-like projections embedded in an outer fibrous layer (Vinson & Scott, 1974).

The eggshells of the endoparasitoids *Nasonia vitripennis*, *Pteromalus puparum* (Linnaeus) and *Catolaccus* spp. (Chalcidoidea, Pteromalidae) resemble each other in having a chorion consisting of three layers: an inner electron-translucent layer and two thin, electron-dense layers. The outermost layer is sometimes spiny (King, Richards & Copland, 1968). Richards (1969) also described the eggshell of *Nasonia vitripennis* but he only reported two layers in the chorion, an inner electron-translucent (EN) and an electron-dense outer (EX) one. The fine regular banding found in the chorion perpendicular to the surface was interpreted as crystalline protein (Richards, 1969). For the fruit-feeding chalcidoid, *Eurytoma amygdali* (Eurytomidae), three chorionic layers were identified in the eggshell, namely an outer columnar layer, a granular layer and an inner translucent layer (Mouzaki & Margaritis, 1994). The

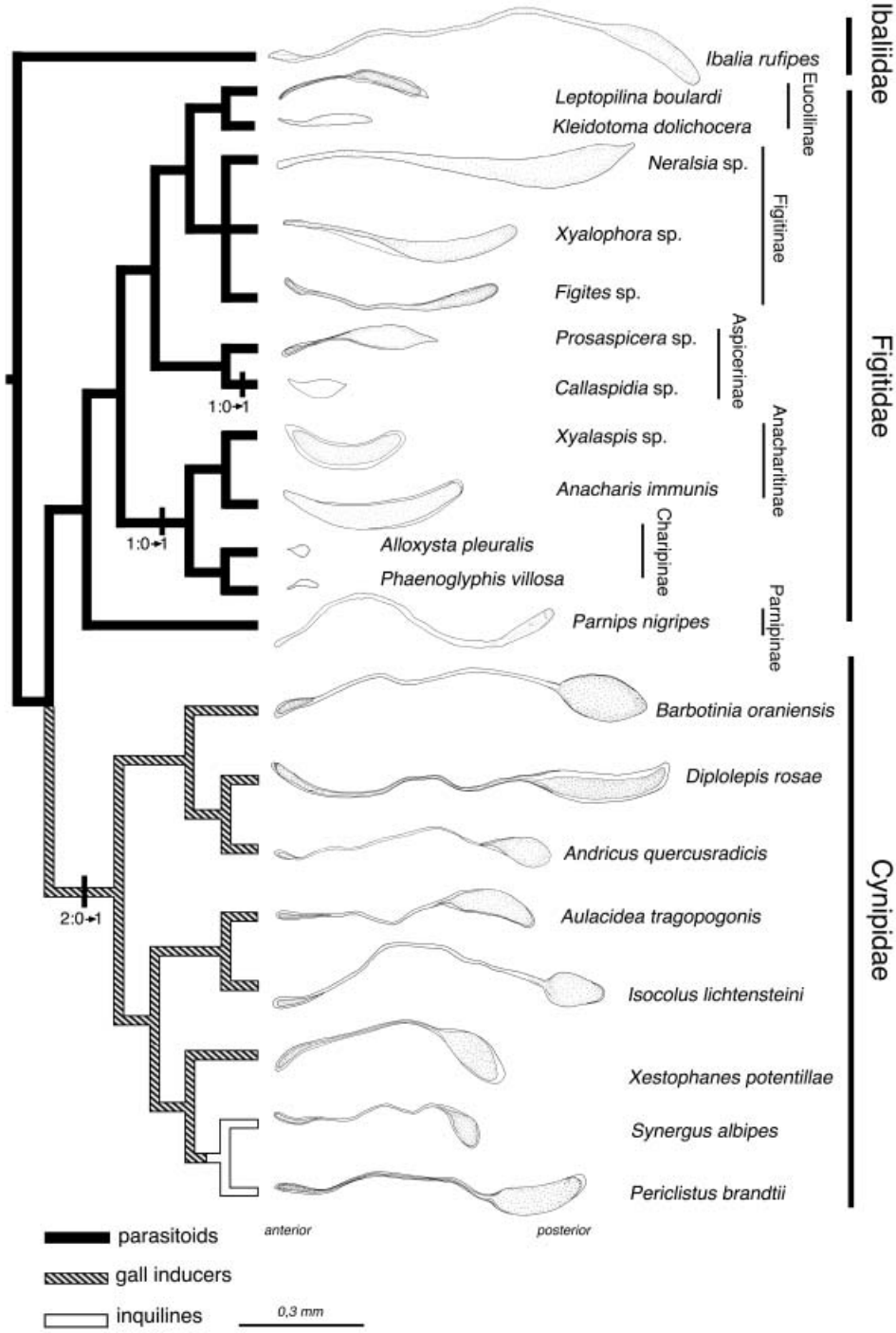

Figure 1. Eggs of 21 cynipoids with a recent estimate of their phylogenetic relationships (Ronquist, 1999). Branch shadings indicate life mode. Character 1 (peduncle) and character 2 (egg shape) (Table 4) are mapped onto the tree. The anterior and posterior poles of the egg are indicated in the figure.

translucent layer probably corresponds to the EN and the other two to the EX.

In this paper, we report the gross egg morphology of a selection of cynipoids representing most of the major evolutionary lineages. We also describe the eggshell ultrastructure of six species: two parasitoids, three gall inducers and one inquiline. The species were chosen to be informative about structural changes in the eggshell associated with the transition from parasitoids to gall inducers, and from gall inducers to inquilines.

MATERIAL AND METHODS

SELECTION OF TAXA

For the study of gross egg morphology, eight species of Cynipidae, 12 species of Figitidae and one species of Ibaliidae were selected.

For examination of eggshell ultrastructure, we chose three species of gall inducers, two parasitoids and one inquiline, indicated by asterisks in Table 1.

Barbotinia and *Parnips*, a gall-inducer and a parasitoid in seed capsule galls of poppies, respectively, have apparently retained many morphological features of the original gall inducers and their parasitic ancestors (Ronquist & Nieves-Aldrey, 2001). Thus, we expected differences between these species to be particularly indicative of changes associated with the origin of gall inducers from parasitoid ancestors. *Xestophanes* belongs to the gall inducers that are most closely related to inquilinous cynipids. Differences between *Xestophanes* and the inquiline *Periclistus* should therefore be informative about changes associated with the origin of inquilines from gall inducers. *Ibalia rufipes* Cresson is a parasitoid from one of the basal cynipoid lineages, and *Diplolepis rosae* (Linnaeus) is an advanced gall inducer, which is unrelated to the inquilines. The classification, biology and collecting sites of the studied species are summarized in Table 1 (see also Fig. 1).

MATERIAL

Eggs for gross morphological studies were dissected from female wasps preserved in 80% ethanol. Eggs for transmission (TEM) and scanning electron microscopy (SEM) were either dissected from females preserved in fixatives (see below) or obtained from infected plant parts immediately after oviposition. Such post-oviposition eggs were available only from three species, namely the gall inducers *Barbotinia oraniensis* (Barbotin) and *D. rosae*, and the inquiline *Periclistus brandtii* (Ratzeburg). The age of dissected females varied. Females of gall inducers and inquilines were reared from galls and killed within a few days of emergence. Females of *Barbotinia* and *Parnips*, however, were kept alive for between 1 and $2^{1}/_{2}$ weeks (for other experiments) before they were killed. Parasitoids were mostly collected in the field, and the age of these females is unknown.

Eggs and females for SEM and TEM were either fixed in AFI solution (18 parts 80% ethanol, one part 34–38% formaldehyde and one part isoacetic acid) or Karnovsky's solution (1/1 mixture of 3% glutaralde-

Table 1. Classification, biology and collecting sites of the species included. The species where the eggshell was examined are marked with an asterisk

Species	Classification	Biology	Collecting site
*Ibalia rufipes**	Ibaliidae	Parasitoid of Siricidae (Hymenoptera)	Sweden, Dalarna
*Parnips nigripes**	Figitidae: Parnipinae	Parasitoid of Cynipidae (Hymenoptera)	Spain, Madrid
Alloxysta pleuralis	Figitidae: Charipinae	Hyperparasitoid of Aphidoidea through Braconidae (Hymenoptera)	Unknown
Phaenoglyphis villosa	Figitidae: Charipinae	Hyperparasitoid of Aphidoidea through Braconidae (Hymenoptera)	Unknown
Anacharis immunis	Figitidae: Anacharitinae	Parasitoid of Hemerobiidae (Neuroptera)	Sweden, Öland
Xyalaspis sp.	Figitidae: Anacharitinae	Parasitoid of Hemerobiidae (Neuroptera)	USA, North Carolina
Prosaspicera sp.	Figitidae: Aspicerinae	Parasitoid of Diptera	Taiwan, Nanto Hsien
Callaspidia sp.	Figitidae: Aspicerinae	Parasitoid of Syrphidae (Diptera)	India, Dehli
Figites sp.	Figitidae: Figitinae	Parasitoid of Diptera	Chile, Lago Chapo
Xyalophora sp.	Figitidae: Figitinae	Parasitoid of Diptera	USA, Texas
Neralsia sp.	Figitidae: Figitinae	Parasitoid of Diptera	USA, Florida
Kleidotoma dolichocera	Figitidae: Eucoilinae	Parasitoid of Diptera	Holland
Leptopilina boulardi	Figitidae: Eucoilinae	Parasitoid of Diptera	Brazil, Campinas
Synergus albipes	Cynipidae: Synergini	Inquiline of Cynipidae on *Quercus*	Sweden, Skåne
*Periclistus brandtii**	Cynipidae: Synergini	Inquiline of Cynipidae on *Rosa*	Sweden, Uppland
*Xestophanes potentillae**	Cynipidae: Aylacini	Gall inducer on *Potentilla*	Sweden, Uppland
Aulacidea tragopogonis	Cynipidae: Aylacini	Gall inducer on *Tragopogon*	Spain, León
Isocolus lichtensteini	Cynipidae: Aylacini	Gall inducer on *Centaurea*	Spain, Cadiz
*Barbotinia oraniensis**	Cynipidae: Aylacini	Gall inducer on *Papaver*	Spain, Madrid
Andricus quercusradicis	Cynipidae: Cynipini	Gall inducer on *Quercus*	Spain, Candelario
*Diplolepis rosae**	Cynipidae: Diplolepidini	Gall inducer on *Rosa*	Sweden, Uppland

hyde and 3% paraformaldehyde). The glutaraldehyde was dissolved in 0.2 M phosphate buffer (pH 7.4) and the paraformaldehyde in pure water by heating to 70 °C, after which the solution was cleared with a few drops of 1 M NaOH. The material was either fixed and stored in Karnovsky's solution or kept in AFI solution for 24 h, and then transferred into 80% alcohol for storage. Before fixation in Karnovsky's solution, the females were tranquillized in carbonated water, which relaxes the body openings and allows the fixatives to enter, penetrating and killing the insect.

For SEM, the eggs were post-fixed in osmium tetroxide, dehydrated in a series of ethanol of concentrations 70–100%, critical point dried, and sputter-coated with a 22-nm-thick gold layer.

For the TEM specimens, the same procedure was followed as for the SEM specimens through to the dehydration in ethanol solutions of increasing concentrations. Then the specimens were transferred to acetone, in which they were kept overnight. Next they were kept for another night in a 1/1 solution of acetone and resin (TAAB 812) followed by embedding in epoxy resin (TAAB 812) and drying for 48 h at 70°C. The specimens were sectioned with a diamond knife microtome (LKB no. 1) in 50-nm-thick sections.

MEASUREMENTS

The length of the egg body is measured from the posterior tip to the transition between the egg body and the peduncle (Fig. 1). The transition between the egg body and the peduncle is sometimes gradual but in all the eggs measured it was possible to discern the point at which the egg body narrows into the peduncle. The width measure is taken at the maximum width of the egg body. The length of the adult female (Table 2) is measured from the head to the posterior tip of the abdomen, excluding antennae and protruding parts of the ovipositor apparatus.

MAPPING OF CHARACTERS

Fitch (unordered) parsimony was used to map egg characters onto a combined phylogenetic estimate (Ronquist, 1999) for the studied taxa.

RESULTS

GENERAL OBSERVATIONS

All studied eggs have a smooth surface unless otherwise described below. Occasionally the egg collapses, giving the surface an irregular appearance. The egg body varies in shape but is always longer than broad.

Table 2. Adult female and egg measurements. Egg load, female length, egg width and length as well as peduncle length are given as average ± standard deviation. The *N* value following each species name signifies the number of eggs measured

Species	Egg load (approx.)	Adult female length (mm)	Egg body width (μm)	Egg body length (μm)	Peduncle length (μm)
Ibalia rufipes (*N* = 10)	180 (*N* = 1)	10.9 ± 0.2 (*N* = 3)	48.3 ± 3.1	280.0 ± 8.2	966.4 ± 171.6
Leptopilina boulardi (*N* = 7)	70 (*N* = 1)	1.5 ± 0.1 (*N* = 3)	27.1 ± 3.9	155.7 ± 14.0	147.1 ± 11.1
Kleidotoma dolichocera (*N* = 7)	80 (*N* = 1)	1.8 ± 0.0 (*N* = 3)	25.7 ± 3.5	138.6 ± 16.5	57.5 ± 33.8
Neralsia sp. (*N* = 8)	40 (*N* = 1)	3.3 ± 0.1 (*N* = 3)	75.6 ± 8.2	328.8 ± 12.8	225.0 ± 35.4
Xyalophora sp. (*N* = 8)	40 (*N* = 1)	3.2 ± 0.1 (*N* = 3)	55.6 ± 5.6	321.3 ± 15.1	182.5 ± 47.7
Figites sp. (*N* = 9)	90 (*N* = 1)	2.9 (*N* = 1)	31.4 ± 6.0	163.9 ± 17.3	180.6 ± 53.1
Prosaspicera sp. (*N* = 10)	30 (*N* = 1)	4.1 ± 0.3 (*N* = 2)	58.0 ± 4.1	186.5 ± 25.2	153.1 ± 26.0
Callaspidia sp. (*N* = 10)	20 (*N* = 1)	3.5 ± 0.4 (*N* = 3)	44.8 ± 3.8	112.0 ± 15.3	31.3 ± 9.5
Xyalaspis sp. (*N* = 7)	20 (*N* = 1)	2.6 ± 0.1 (*N* = 3)	55.0 ± 6.5	276.4 ± 17.0	No peduncle
Anacharis immunis (*N* = 8)	20 (*N* = 1)	2.8 ± 0.4 (*N* = 2)	57.2 ± 4.1	438.8 ± 27.1	No peduncle
Alloxysta pleuralis (*N* = 5)	30 (*N* = 1)	1.2 ± 0.1 (*N* = 3)	28 ± 4.2	53.0 ± 4.5	Indistinct peduncle
Phaenoglyphis villosa (*N* = 8)	80 (*N* = 1)	1.3 ± 0.1 (*N* = 3)	19.1 ± 1.9	60.6 ± 6.8	Indistinct peduncle
Parnips nigripes (*N* = 10)	83 (*N* = 1)	3.2 ± 0.5 (*N* = 4)	30.0 ± 4.7	159.0 ± 11.2	520.0 ± 5.8
Barbotinia oraniensis (*N* = 12)	200 (*N* = 1)	3.3 ± 0.1 (*N* = 2)	90.8 ± 12.3	193.8 ± 11.6	564.0 ± 83.1
Diplolepis rosae (*N* = 11)	408 (*N* = 5)	3.7 ± 0.4 (*N* = 3)	58.6 ± 6.7	317.7 ± 17.9	916.9 ± 335.9
Andricus quercusradicis (*N* = 9)	>1000 (*N* = 1)	4.1 ± 0.3 (*N* = 3)	68.9 ± 2.2	121.1 ± 7.8	778.0 ± 173.2
Aulacidea tragopogonis (*N* = 8)	40 (*N* = 1)	2.0 ± 0.1 (*N* = 3)	55.0 ± 3.8	202.2 ± 12.4	426.0 ± 16.7
Isocolus lichtensteini (*N* = 8)	200 (*N* = 1)	3.0 ± 0.3 (*N* = 3)	83.8 ± 7.4	148.1 ± 4.6	623.0 ± 46.3
Xestophanes potentillae (*N* = 12)	88 (*N* = 6)	2.3 ± 0.5 (*N* = 3)	65.8 ± 4.9	184.6 ± 8.6	380.0 ± 25.4
Synergus albipes (*N* = 7)	35 (*N* = 1)	1.5 ± 0.2 (*N* = 3)	46.4 ± 2.8	100.0 ± 5.8	352.5 ± 66.0
Periclistus brandtii (*N* = 10)	73 (*N* = 5)	2.6 ± 0.1 (*N* = 3)	62.3 ± 7.8	220.3 ± 7.9	529.0 ± 49.9

Most eggs have a peduncle, the length of which varies greatly between species (Table 2). It is, however, with few exceptions longer than the egg body, ending in an elongate swelling (Fig. 1). The number of eggs per female varies among and within species (Table 2). The egg size does not always vary according to adult female size. This variation is interspecific as the egg size is fairly constant within the species (Table 2).

SEM AND LIGHT MICROSCOPE (LM) OBSERVATIONS

The gross morphology of all studied eggs is illustrated in Figure 1.

Ibalia rufipes
A single female of this species carried 180 eggs. This species is considerably larger than the other species, and its eggs are also among the largest. The egg body is relatively narrow, almost six times as long as broad, and tapers into a long peduncle, which is more than three times longer than the egg body.

Alloxysta pleuralis (Cameron)
A single female of this species carried about 30 eggs. The egg is very small. The egg body is less than twice as long as wide. The shape of the egg body is oval, and it has an inconspicuous peduncle.

Phaenoglyphis villosa (Hartig)
A single female of this species carried about 80 eggs. The egg is very small. The egg body is about three times longer than wide. The shape of the egg body is slightly elongate, and it has an inconspicuous peduncle.

Anacharis immunis Walker
A single female of this species carried about 20 eggs. The egg body is almost eight times longer than wide. The shape of the egg body is elongate and sligthly curved, and there is no distinct peduncle.

Xyalaspis petiolata Kieffer
A single female of this species carried about 20 eggs. The egg body is about five times longer than wide. The shape of the egg body is elongate and slightly curved, and there is no distinct peduncle.

Prosaspicera sp.
A single female of this species carried about 30 eggs. The egg body is slightly more than three times longer than wide. The egg body is ellipsoid with a pointed posterior tip, and the peduncle is slightly shorter than the egg body.

Callaspidia sp.
A single female of this species carried about 20 eggs. The egg body is slightly less than three times longer than wide. The egg body is elongate with a rounded tip. The peduncle is only a third of the length of the egg body.

Figites sp.
A single female of this species carried about 90 eggs. The egg body is more than five times longer than wide. The shape of the egg body is elongate and rounded. There is no sharp transition point between the egg body and the peduncle, which is slightly longer than the egg body.

Xyalophora sp.
A single female of this species carried about 40 eggs. The egg body is sligthly less than six times longer than wide. The shape of the egg body is elongate and rounded, and there is no sharp transition point between the egg body and the peduncle. The peduncle is sligthly more than half the length of the egg body.

Neralsia sp.
A single female of this species carried about 40 eggs. The egg body is more than four times longer than wide. The shape of the egg body is elongate with a pointed end. The egg body tapers gradually into the peduncle. The peduncle is a little shorter than the egg body.

Kleidotoma dolichocera Thomson
A single female of this species carried about 80 eggs. The egg body is more than five times longer than wide. The narrow egg body is elongate with a short peduncle (less than half the length of the egg body).

Leptopilina boulardi (Barbotin, Carton & Kelner-Pillault)
A single female of this species carried about 70 eggs. The egg body is almost six times longer than wide. The egg body is elongate and slightly pointed. The peduncle is almost as long as the egg body.

Parnips nigripes (Barbotin)
A single female of this species carried 83 eggs. The egg is narrow and tapers gradually, without any marked

transition, into the peduncle. The egg body is more than five times as long as broad, and the peduncle is slightly more than three times longer than the egg body.

Barbotinia oraniensis
A single female of this species carried 200 eggs. The egg body is oval and rounded at the posterior end. The transition between egg body and peduncle is sharp. The egg body is more than twice as long as wide, and the peduncle is about three times longer than the egg body but this varies between specimens.

Diplolepis rosae
Females carry on average 408 eggs ($N = 5$, range 340–490). The egg is elongate and slender. The egg body is more than five times as long as broad. The peduncle length varies enormously between individual eggs from one and a half to four times the length of the egg body (Table 2).

Andricus quercusradicis (Fabricius)
A single female of this species carried more than 1000 eggs. The egg body is slightly less than twice as long as wide. The egg body is oval, slightly narrower towards the posterior end. The transition point between the egg body and peduncle is sharp. The peduncle is more than six times longer than the egg body.

Synergus albipes Hartig
A single female of this species carried around 35 eggs. The egg body is about twice as long as wide. The egg is distinctly divided into an oval egg body and a slender peduncle, which is three and a half times longer than the egg body.

Periclistus brandtii
Females of this species carry on average 73 eggs ($N = 5$, range 50–98). The egg is banana-shaped and distinctly divided into an egg body and a long and narrow peduncle. The egg body is more than three times as long as wide, and the peduncle is at least twice as long as the egg body.

Xestophanes potentillae (Retzius)
Females of this species carry on average 88 eggs ($N = 6$, range 24–164). The egg body is oval and a little less than three times as long as wide. The peduncle is twice the length of the egg body. We were unable to study the chorion surface of this egg with SEM because the maternal tissue did not separate easily from the chorion.

Aulacidea tragopogonis (Thomson)
A single female of this species carried about 40 eggs. The egg body is three and a half times longer than wide. The egg is distinctly divided into an elongate egg body and a slender peduncle, which is about twice the length of the egg body.

Isocolus lichtensteini (Mayr)
A single female of this species carried about 200 eggs. The egg body is less than twice as long as wide. The egg body is oval and slightly narrower towards the posterior end. The transition point between the egg body and peduncle is sharp. The peduncle is almost twice as long as the egg body.

TEM OBSERVATIONS

General observations
The cynipoid chorion consists of two layers, which will be referred to as the endochorion (EN) and the exochorion (EX) (Fig. 2). The VE is not as distinct as the EX and EN, and is only occasionally referred to here. EX tends to separate easily from EN in most of the studied gall inducers and the inquiline species.

Ibalia rufipes (Fig. 2)
The EX consists of an outer part containing crystalline structures of various sizes, and an inner part, which is homogenous and electron-dense. The EN is of equal thickness as the EX, homogenous and electron dense. This layer is flexible and does not preserve the round shape of the egg during fixation and embedding, which it does in most of the other species.

Parnips nigripes (Figs 2, 4, 5)
The EX consists of two layers, which are clearly separated. The outer part is filled with small packages or bundles of crystalline elements. Within each package the elements are orientated in parallel but the orientation differs between the packages (Fig. 2). The inner part of the EX is relatively uniform and does not contain any crystalline elements. The EN is slightly more than half as thick as the EX, homogeneous and flexible. It is closely attached to the EX but separates easily from the VE. The cross-section of this egg is irregular rather than circular or oval in outline, which we interpret as fixation artefacts.

Periclistus brandtii (Figs 2, 3)
The EX is divided into two parts. The outer part contains closely packed crystalline rods arranged in rows that are perpendicular to the surface of the egg. The inner part of the EX is less electron-dense (Fig. 2). It

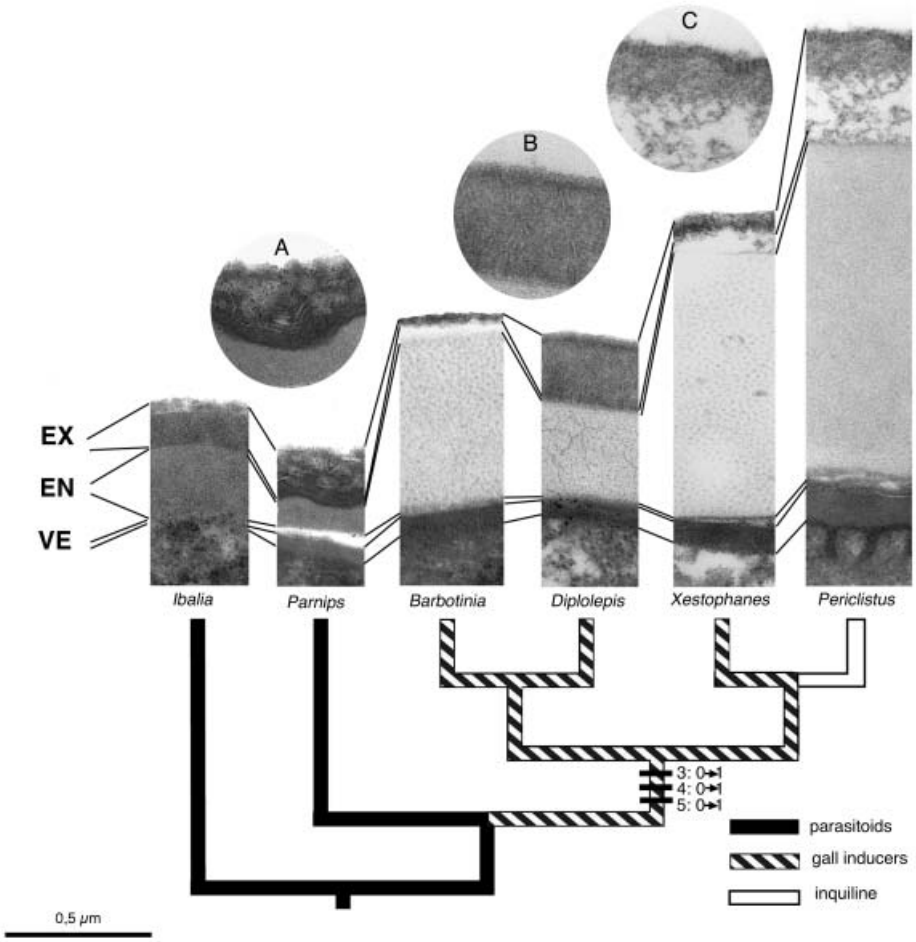

Figure 2. Transmission electron micrographs of cross-sections of six cynipoid eggs. Phylogenetic relationships (Ronquist, 1999) are indicated at the bottom. The branch shadings indicate life mode and the inserts are greater magnifications of the exochorion of (A) *Parnips nigripes*, (B) *Diplolepis rosae* and (C) *Periclistus brandtii* showing the three main arrangements of crystalline elements in this layer. The egg characters 3 (eggshell thickness), 4 (endochorion texture) and 5 (crystalline element arrangements) (Table 4) are mapped onto the appropriate branches. In some of the sections what appears to be an extra layer between EX and EN (in *Barbotinia, Xestophanes* and partly in *Periclistus*) and between EN and VE (in *Parnips*) are artefacts as the layers often separate easily from each other. In *Periclistus*, however, the EX appears to consist of an outer part that is more compact than the inner part.

also contains irregularly spaced crystalline elements. The EN is electron-translucent, uniform in structure and about ten times thicker than the EX (Table 3). The VE seems to be anchored in the oocyte by extensions (Fig. 2)

After oviposition, the outer part of the EX is strongly compressed, and the inner layer of the EX is more homogeneous than it was before oviposition. Towards the anterior end of the egg the EX and EN contain vesicle-like structures (Fig. 3). The vesicles are electron-translucent in the EX and electron-dense in the EN. The EN is less homogeneous than before oviposition, and gradually becomes more electron-dense towards the VE.

Xestophanes potentillae (Fig. 2)

The EX is not divided into sublayers. It is always closely associated with maternal tissue. Electron-translucent crystalline rods perpendicular to the layer underneath can be seen to be more or less evenly distributed in rows. The EN is electron-translucent, homogenous and about 15 times the thickness of the EX.

Barbotinia oraniensis (Figs 2, 5)

The EX is not divided into sublayers. It was disassociated from the inner layers in the eggshell of the sections, presumably an effect of fixation. The crystalline

Table 3. Eggshell measurements. The measurements were performed on the TEM micrographs. They were made close to the middle of the egg body of pre-oviposition eggs, and are given as mean ± standard deviation

Species	Endochorion (EX) (nm)	Exochorion (EX) (nm)	Chorion (EN + EX) (nm)
Ibalia rufipes	192.1 ± 24.7	176.5 ± 38.3	368.5 ± 47.8
Parnips nigripes	122.3 ± 30.4	209.8 ± 47.6	332.2 ± 72.8
Periclistus brandtii	1136.2 ± 38.9	119.5 ± 55.2	1255.7 ± 51.4
Xestophanes potentillae	893.1 ± 31.8	59.04 ± 17.9	952.2 ± 46.7
Barbotinia oraniensis	585.9 ± 135.6	45.0 ± 15.0	630.9 ± 143.7
Diplolepis rosae	391.3 ± 43.0	259.4 ± 18.0	650.7 ± 55.5

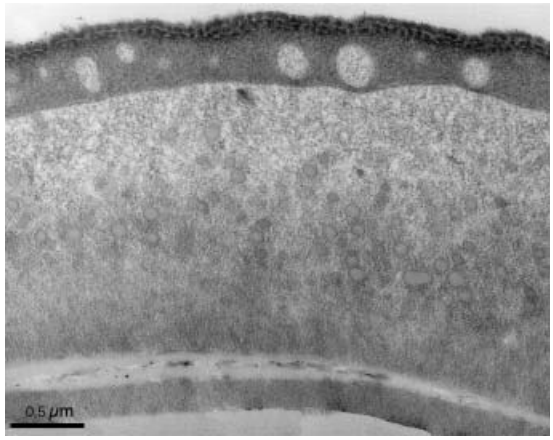

Figure 3. Cross-section of the eggshell of the peduncle of *Periclistus brandtii* after oviposition, showing vesicle-like structures in the EX and EN.

elements in the EX are indistinct. They appear to be arranged in parallel, perpendicular to the EN. The EN is homogeneous, electron-translucent and about 14 times the thickness of the EX.

The eggshell does not change much during oviposition, except that the EX becomes firmly associated with the EN. Crystalline elements are not visible. The EN is unchanged from the pre-oviposition egg, except for the occurrence of dark elongate structures towards the inner part of the layer, which are probably vesicles originating due to stretching effects.

Diplolepis rosae (Figs 2, 4)

The EX is not divided into sublayers. It is an electron-dense layer containing electron-translucent crystalline rods, which are shorter than the thickness of this layer. The cystalline rods are scattered irregularly in the EX but they are all directed perpendicularly to the layer underneath (Fig. 2). A striking feature is that a narrow region of the eggshell at the posterior (non-pedunculate) end of the egg has a less dense and apparently porous EX (Fig. 4). The EX is always

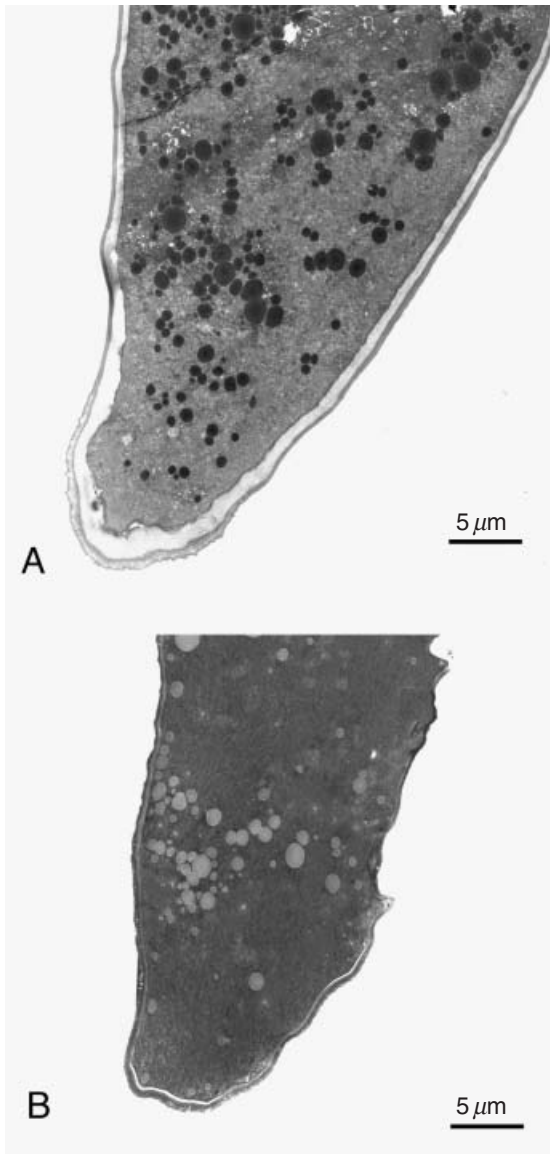

Figure 4. Longitudinal section of posterior tip of eggs of (A) *Diplolepis rosae* and (B) *Parnips nigripes*, illustrating the EX modification of *D. rosae* in this region.

Table 4. Possible evolutionary changes between the parasitoid life mode, the gall inducers and the inquilines

Character	Ancestral state (0)	Derived state (1)
1. Peduncle	Present	Absent
2. Egg shape	Narrow, gradual transition between eggbody and peduncle	Broad, marked transition between egg body and peduncle
3. Eggshell	Thin	Thick
4. Endochorion	Flexible	Rigid
5. Crystalline elements	Different directions	In parallel

closely attached to the EN. The EN is more electron-translucent than the EX, and about twice as thick (Table 3).

After oviposition, the eggshell seems to have weakened. It no longer maintains the rounded–oval form of the egg but is irregular in outline. Apart from this, the layers look more or less the same as in the pre-oviposition egg. No stretching effects or vesicle-like structures can be seen.

Mapping of selected egg characters on to the phylogeny of Cynipoidea

Based on the observations, we could distinguish and map the evolution of five discrete characters (Table 4, Figs 1, 2). The mapped characters only require one or two steps each, which is quite conservative.

DISCUSSION

Egg shape

Previous studies in which cynipoid eggs have been discussed largely confirm the observations reported here. James (1928) illustrated the egg of *Figites anthomyiarum* Bouché (Figitidae), which, apart from a constriction in the middle of the egg body, is very similar to the *Figites* egg examined here. A few other cynipoid parasitoid eggs have been described; in these, the egg shape varies from almost circular with a strongly reduced peduncle in *Alloxysta* sp. (Figitidae: Charipinae) (described under the name *Charips* sp., Haviland, 1921) to more elongate with peduncles of varying length (Figitidae: Eucoilinae) (Huzimatu, 1940; Jenni, 1951; Wishart & Monteith, 1954). Again, these results agree with those presented here.

A few descriptions of eggs of gall inducers and inqulines (Cynipidae) show similar variation in gross shape as that observed among the species studied here. The eggs were described as short and oval in the oak gallers *Plagiotrochus suberi* Weld (Diaz, 1972), *Biorhiza pallida* (Olivier) (Frühauf, 1924) and the oak inquiline *Synergus pacificus* McCracken and Egbert (Evans, 1965) and more elongate in the rose galler *Diplolepis rosae* (Frühauf, 1924; Schröder, 1967; Bronner, 1985). All of these papers document the distinct division into a narrow peduncle and a broader egg body that we found in all the cynipid eggs examined by us.

We found two qualitative differences in egg shape among the examined cynipoids. The first one concerns the peduncle (Table 4, character 1). Cynipoid eggs generally have a long and distinct peduncle but the peduncle is either strongly reduced or totally missing in the eggs of Charipinae (*Alloxysta pleuralis* and *Phaenoglyphis villosa*), Anacharitinae (*Anacharis immunis* and *Xyalaspis* sp.) and one of the examined Aspicerinae species (*Callaspidia* sp. but not *Prosaspicera* sp.). Mapping of this character onto the cynipoid phylogeny indicates that presence of a long peduncle is the ancestral state for the Cynipoidea, and that the reduction of the peduncle occurred twice, once in the common ancestor of the Charipinae and Anacharitinae, and once within the Aspicerinae (*Callaspidia*) (Fig. 1). Anacharitines and charipines have previously been placed in different families and were first suggested to form a monophyletic group by Ronquist (1999) based on characters in the adult morphology, mainly ovipositor features. The reductions of the peduncle reported here thus provide further support for the monophyly of this grouping.

The second qualitative difference in egg shape concerns the division of the egg into an egg body and a peduncle (Table 4, character 2). We found that parasitoids in general have small and narrow eggs, which taper gradually into a relatively wide peduncle, whereas gall inducers and inquilines have eggs, which are distinctly divided into a massive egg-body and a thin and elongate peduncle. Mapping of the character egg shape on the phylogenetic tree (Fig. 1) shows that it is the parasitoid state that is ancestral. Apparently, it is the egg body that has increased in size in the common ancestor of the Cynipidae. This is perhaps most clearly illustrated by a comparison of the eggs of *P. nigripes* and *B. oraniensis*, a parasitoid and a gall inducer of about the same body size and with ovipositors of approximately equal dimensions. The eggs are of similar length, yet the egg body of

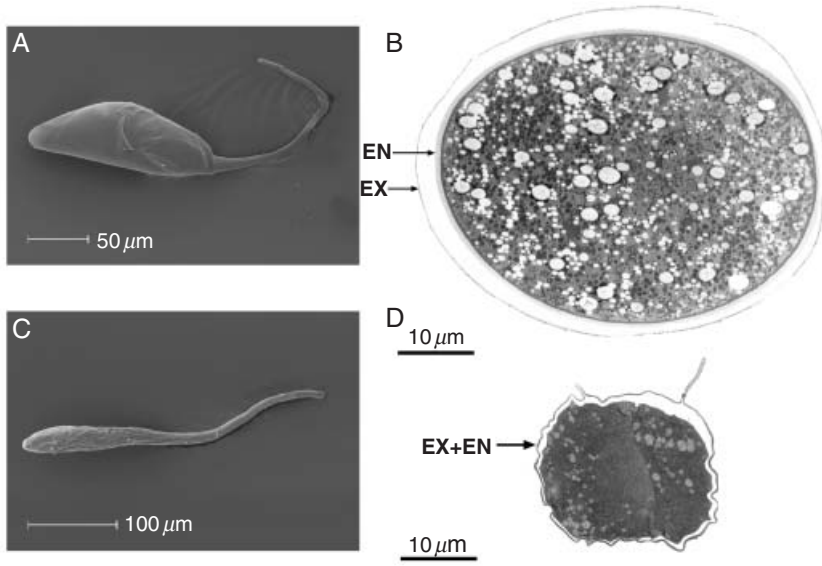

Figure 5. Eggs of the gall inducer *Barbotinia oraniensis* (A, B) and the parasitoid *Parnips nigripes* (C, D). The SEM micrographs (A, C) illustrate the general shape of the egg, and TEM micrographs (B, D) illustrate the great difference in eggshell thickness between the two species.

P. nigripes is only 30 μm wide whereas the egg body of *B. oraniensis* is about three times as wide (90.8 μm) (Fig. 5).

The narrow parasitoid egg presumably passes more rapidly through the egg channel of the terebra. Shortening oviposition times may be important to avoid live hosts escaping the oviposition attempt, and the narrow eggs may thus be an adaptation to the insect-parasitic life style. The fact that the parasitoid eggs are probably hydropic (shown for Ibaliidae (*Ibalia*; Chrystal, 1930; Flanders, 1942) and Figitidae (Eucoilinae: *Leptopilina*; Quicke, 1997)), i.e. they absorb nutrients from the host, means that they need little yolk and can be rather small.

The eggs of the gall-inhabiting Cynipidae, by contrast, are undoubtedly anhydropic, contain more yolk and must therefore be larger. Another factor that probably contributes to this effect is that the gall-inhabiting larvae probably go through an initial phase in their development, during which they cannot feed but instead spend substantial energy on redirecting plant development into forming or reshaping a gall. Thus, it seems likely that the egg of the gall inhabitants must contain more energy reserves, and be larger, than the egg of the parasitoids. If the maximum length of the egg is constrained, for instance by mechanical factors related to oviposition, then the drive towards heavier eggs may have resulted in a more distinct division into a large egg body and a thin peduncle. As mentioned above, the comparison between *Parnips* and *Barbotinia* does suggest that it is, indeed, the egg body that has increased in size in the gall inhabitants. This hypothesis can be tested by comparing egg yolk contents between gall inhabitants and parasitoids.

The broadening of the egg in the cynipid ancestor may also be related to an increased risk of desiccation. The parasitoid egg is normally deposited within the body of the host, where it does not suffer from dehydration. However, the egg of gall inhabitants is usually attatched to the surface of the plant tissue and therefore needs to be much more drought tolerant. A round egg has a lower surface-to-volume ratio than a sausage-shaped egg and this may also have contributed to the change towards more globular egg bodies in the gall inhabitants, despite the increased stress during oviposition and the extended oviposition times.

EGG LOAD

The egg load varies considerably between species (Table 2), and the size of the egg load might be indicative of whether a species is synovigenic, providing eggs continuously, or pro-ovigenic, emerging with all eggs mature. Like most other koinobionts (Quicke, 1997), cynipoid parasitoids are probably pro-ovigenic. The large egg loads of newly emerged gall wasps indicate that they are pro-ovigenic too. However, some species may be pro-synovigenic, which means that their females emerge with a good number of mature eggs but can mature more eggs as their original supply of mature eggs becomes depleted (Quicke, 1997). This appears to be the case for the rose galler *Diplolepis rosae*, for which a greater number of eggs were

counted in 5–7-day-old females than for freshly emerged females (Schröder, 1967).

Egg surface structures

Leuckart (1855) believed that he had found three very fine micropyles in the egg of the gall wasp *Cynips quercus* (Fourcroy) (Cynipoidea: Cynipidae), situated in the peduncle close to the transition zone between the egg body and the peduncle. Wishart & Monteith (1954), however, claimed that the micropyle is at the tip of the peduncle of *Trybliographa rapae* (Westwood) (Cynipoidea: Figitidae) and that it only has one channel leading into the egg. We were not able to observe the micropyle in any of the six cynipoid species studied with SEM and TEM, nor were we able to section the tip of the peduncle or study it with SEM. Therefore we cannot determine which one of these two positions is the common micropyle position in cynipoids. We did not observe any aeropyles or other major structures on the surface of the egg, except for the specialized tip of the *D. rosae* egg (see below).

The eggshell

There are striking differences among cynipoids in the thickness of the eggshell. The chorion is much thinner in the eggs of the two parasitoid species (*Parnips nigripes* and *Ibalia rufipes*) than in the gall-inhabiting species (Table 3). Phylogenetic mapping of this trait suggests that the eggshell became thicker in the ancestor of the gall inhabitants (Table 4, character 3; Fig. 2). Presumably, the thin eggshell of the parasitoids is related to the fact that these eggs are deposited inside the hosts and that they are hydropic.

The chorion of hydropic eggs of other parasitic wasps is known to be highly convoluted and flexible, unlike the rigid and smooth chorion of anhydropic eggs (Quicke, 1997).

Of the layers in the chorion, the EX varies most in structure in cynipoids (Fig. 2). In the pre-oviposition eggs it is often closely attatched to layers of maternal cells, which are sometimes difficult to separate from the egg (*Xestophanes*). Among the six species we examined for eggshell structure, *D. rosae* is the only species that lays eggs in a somewhat exposed environment, i.e. between leaves of young buds of *Rosa* sp. It is also the only species that has a thick electron-dense EX (Table 3) (except for the posterior part of the egg, see below). The EX is thin in the other examined species but it nevertheless constitutes a large proportion of the total eggshell thickness in *P. nigipes*.

In most of the examined species, the EX contains electron-translucent rods, which presumably represent protein crystals (Fig. 2). In the gall-inhabiting species, the rods have the same size and structure and are arranged in rows that are perpendicular to the surface of the egg. This is not the case in the parasitoids. In *P. nigripes*, the crystalline structures are packed into stacks and the orientation of the stacks is variable (Fig. 2A). In the other examined parasitoid, *Ibalia rufipes*, the rods also varied in shape and orientation, although they did not seem to be arranged in stacks.

Phylogenetic mapping suggests that the regularly arranged crystal rods of gall inhabitants constitute a synapomorphy for them (Fig. 2). The functional significance of this character is unclear. Sahlén (1994a) suggested that a *Parnips*-like arrangement of crystalline elements in the EX of Odonata eggs gives elasticity to the layer, an important property for a hydropic egg.

In all the species studied here, the EN is the thickest eggshell layer and it is rather uniform in texture. It is a translucent, gelatinous layer, which is mostly homogenous but sometimes contains electron-dense spots. It lacks both the typical banding of crystalline proteins seen in the eggs of many insects (Furneaux & Mackay, 1972) and the sublayers reported in eggs of Odonata and Diptera by Sahlén (1994a,b, 1995a,b). In the three gall wasp species (*B. oraniensis, D. rosae* and *X. potentillae*) and the inquiline (*P. brandtii*), the EN is rigid and gives the cross-section of the egg a rounded outline. In the parasitoids (*P. nigripes* and *I. rufipes*), by contrast, the EN is both thinner and more flexible (Table 3). Mapping of this trait onto the cynipoid phylogeny suggests that the rigid endochorion is the derived state, and that this state evolved in the ancestor of the gall inhabitants (Table 4, character 4; Fig. 2).

We did not measure the thickness of the VE because it is not well defined in all the species but in general it is thinner than the EX. In the inquiline *P. brandtii,* the VE is anchored to the oocyte with extentions (Fig. 2). Similar VE anchors have also been observed in the chalcidoids *Nasonia vitripennis* and *Catolaccus* sp. (King *et al*., 1968), where they were suggested to be a protective device for the oocyte during oviposition when the chorion often separates from the VE and moves relative to it (Sahlén, 1994a).

Eggshell changes during oviposition

In cynipoids, the entire egg passes through the narrow egg channel of the terebra during oviposition. The posterior end of the egg (the egg body) is first pressed into the basal section of the egg canal, while the contents of the egg are pressed into the peduncle. Back-and-forth movements of the first and second valvulae and spine-like scales lining the interior of the egg canal (Austin & Browning, 1981) force the egg down the terebra and once the egg body starts to protrude from the apex of the terebra, the egg content starts to flow back into the

egg body from the peduncle (Frühauf, 1924; Bronner, 1985). Thus the eggshell is exposed to enormous mechanical stress during oviposition.

Because the egg body and peduncle are exposed to different forces during oviposition, one might expect to see some structural differences between them. The walls of the peduncle must be highly elastic and expandable to allow egg content to temporarily fill the peduncle, making the egg body narrow enough to pass through the ovipositor. The egg body, by contrast, must be able to contract to initiate the flow of egg content into the peduncle, and to resume its original shape after oviposition. However, we observed only minor structural differences between the peduncle and the egg body chorion. The EX of the peduncle is more compact than it is in the rest of the egg and it is also more firmly attatched to the EN. The EN is in most cases thicker and the VE is often more irregular there than in other parts of the egg.

The mechanical stress during oviposition could also have some lasting impact on the eggshell. We were able to test this idea by comparing newly laid eggs with pre-oviposition eggs in three gall-inhabiting species: *Periclistus brandtii*, *Barbotinia oraniensis* and *Diplolepis rosae.* In two of these, *Periclistus brandtii* and *Barbotinia oraniensis*, we observed obvious structural changes. In the pre-oviposition eggs, the boundary between the EN and EX was clear and the two layers were homogeneous. In the post-oviposition eggs, however, the EX contained numerous small vesicles of similar electron density as the EN. Similarly, the EN contained vesicles of similar electron density as the EX. We interpret these vesicles as the result of minor cracks in the EN and EX boundary during oviposition, as the result of mechanical stress, permitting some exchange of EN and EX material. The vesicles were visible throughout the eggshell but were particularly abundant in the peduncle (Fig. 3).

Communication through the eggshell

If the egg plays a major role in the gall-induction process, and gall induction is due to chemical signals produced by the gall wasp, then the eggshell should be equipped with special pores or other structures permitting chemical exchange between the embryo and its environment. However, we observed specialized eggshell structures only in one of the four examined gall-inhabiting species, namely *Diplolepis rosae*. Whereas the eggshell was uniform around the egg body in all other examined species, *D. rosae* had a specialized region at the posterior end of the egg where the eggshell was distinctly more electron-translucent than elsewhere (Fig. 4). This apparently porous region of the eggshell corresponds to the portion of the egg that is inserted into the epidermis of the host plant leaf during oviposition. It can be seen in the egg both before and after oviposition. Bronner (1985) noted this specialized posterior region of the *D. rosae* egg, and suggested that it serves as an anchor. It is also possible that it facilitates the exit of the young larva. However, considering the fact that the development of the host leaf is significantly affected after oviposition but before the larva hatches in *D. rosae* (Magnus, 1914), it also appears possible that the porous region allows passage of chemical compounds from the egg into the host plant. It is a hypothesis that merits further attention.

CONCLUSIONS

Our studies show several major changes in egg morphology in the Cynipoidea of both functional and phylogentic significance. The transition from parasitoids to gall inducers, in particular, has been associated with many changes in egg morphology, whereas the shift from gall inducers to inquilines has not. We have shown that there is a specialized egg region that may permit chemical communication between the embryo and the host plant in the gall inducer *Diplolepis rosae* but the lack of such structures in other gall inhabitants may indicate that the embryo inside the egg does not generally play a major role in inducing gall development in cynipids.

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