Small Is Beautiful: Features of the Smallest Insects and Limits to Miniaturization

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Abstract

Miniaturization leads to considerable reorganization of structures in insects,
affecting almost all organs and tissues. In the smallest insects, comparable
in size to unicellular organisms, modifications arise not only at the level of
organs, but also at the cellular level. Miniaturization is accompanied by allo-
metric changes in many organ systems. The consequences of miniaturization
displayed by different insect taxa include both common and unique changes.
Because the smallest insects are among the smallest metazoans and have the
most complex organization among organisms of the same size, their pecu-
liar structural features and the factors that limit their miniaturization are of
considerable theoretical interest to general biology.

6.1

INTRODUCTION

Miniaturization:
extreme phylogenetic
size decrease or
evolution of extremely
small body size within
a lineage (see
Reference 36)

**Microinsects:** insects
with adult body sizes
smaller than 2 mm

Miniaturization is a widespread trend in animal evolution (36) and one of the principal directions of evolution in insects (10). As a result, some insects are comparable in size to unicellular organisms (**Figure 1**). Body size, especially when extremely small, largely determines the morphology, physiology, and biology of animals (77).

The first specialized studies on the effects of size on structure in insects were published almost 70 years ago (74, 30). They were later followed by some structural studies on larvae of minute insects (4, 33). Large-scale work on the influence of miniaturization on structure in insects started only a decade ago (5, 64) and has developed into a field of study represented by more than 15 publications annually (**http://thomsonreuters.com/thomson-reuters-web-of-science/**). Miniaturization-related patterns have recently been discussed not only in publications on morphology but also in those on taxonomy (e.g., 32, 39). Remarkably, a new area is currently forming within this field: the study of the central nervous system in microinsects, largely based on the recently discovered miniaturization-related morphological patterns of its structure (12, 14, 23, 91).

The smallest insects are the wingless males of the egg parasitoid *Dicopomorpha* (Mymaridae); the smallest flying insects are wasps of the genera *Kikiki* (Mymaridae) and *Megaphragma* (Trichogrammatidae); and the smallest nonparasitic insects are beetles of the genera *Nanosella* and *Scydosella* (Ptiliidae) (**Table 1**). The smallest insects are among the smallest metazoans; only some members of a few other invertebrate taxa are even smaller (52).
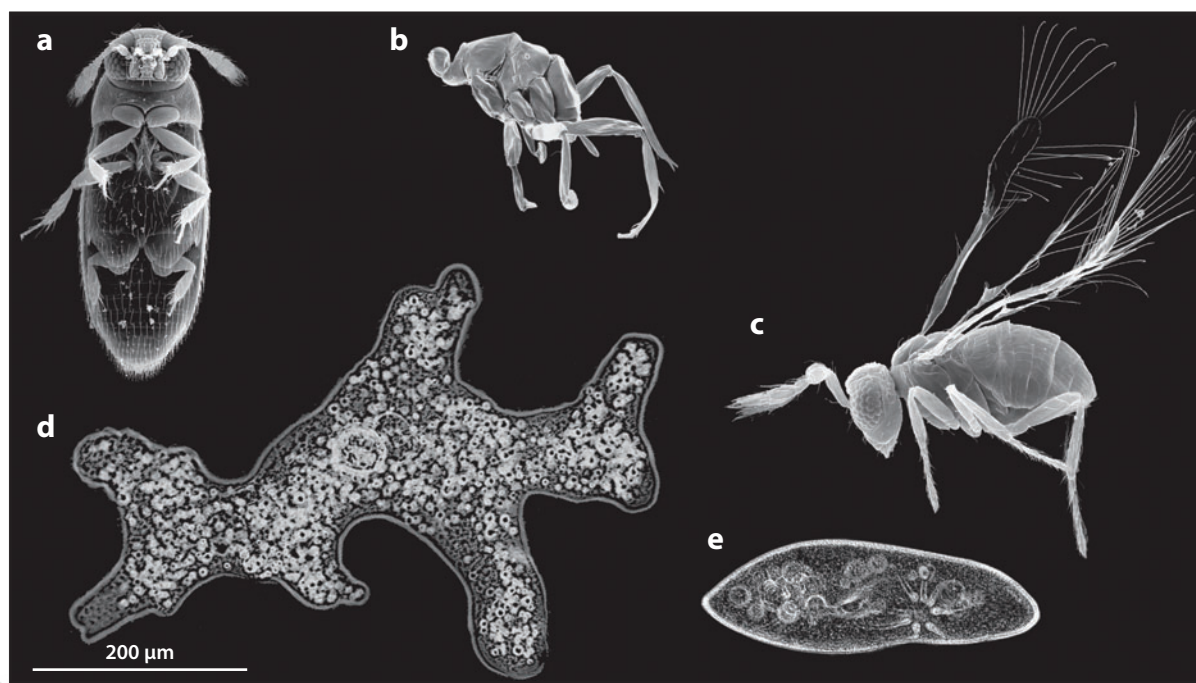
Figure 1

Relative body sizes of the smallest insects and unicellular organisms: (*a*) *Nanosella* sp., (*b*) *Dicopomorpha echmepterygis* (modified from 38), (*c*) *Megaphragma mymaripenne*, (*d*) *Amoeba proteus*, (*e*) *Paramecium caudatum*. Panels *c–e* modified from Reference 68.

Table 1   Insects with a body length of 300 μm or less

Species	Body length (μm)	Family	Reference
Dicopomorpha echmepterygis Mockford, 1997	139 (male)	Mym.	55
Kikiki huna Huber, Beardsley, 2013	160 (female)	Mym.	39
Megaphragma caribea Delvare, 1993	170	Tri.	16
Kikiki huna Huber & Beardsley, 2000	190	Mym.	37
Alaptus borinquensis Dozier, 1932	200 (male)	Mym.	39
Tinkerbella nana Huber & Noyes, 2013	210 (male)	Mym.	39
Alaptus magnanimus Anandale, 1909	210	Mym.	7
Megaphragma amalphitanum Viggiani, 1997	225	Tri.	86
Megaphragma longiciliatum Subba Rao, 1969	250	Tri.	81
Megaphragma mymaripenne Timberlake, 1924	252[a]	Tri.	84
Megaphragma deflectum Lin, 1992	260	Tri.	45
Megaphragma striatum Viggiani, 1997	270 (male)	Tri.	85
Megaphragma decochaetum Lin, 1992	280	Tri.	45
Megaphragma stenopterus Lin, 1992	280	Tri.	45
Megaphragma ghesquiere Ghesquière, 1939	290	Tri.	29
Scydosella musawasensis Hall, 1999	300	Pti.	35
Nanosella spp.	300	Pti.	2
Megaphragma macrostigmum Lin, 1992	300	Tri.	45

Abbreviations: Mym., Mymaridae; Pti., Ptiliidae; Tri., Trichogrammatidae.

[a]Dozier (18) gives 172 μm as the body length of *Megaphragma mymaripenne*, but this measurement probably refers to *Megaphragma caribea*.

MORPHOLOGICAL EFFECTS OF MINIATURIZATION ON DIFFERENT ORGAN SYSTEMS

Cuticular Structures (Integument, Skeleton, and Appendages)

The principal structure of the integument is rather similar in microscopic and larger insects, but in the former the cuticle is considerably thinner (64–66). For example, in one of the smallest beetles, the ptiliid *Mikado*, the average cuticle thickness is 1.9 μm in adults and even thinner in first instar larvae: 0.68 μm (70). At the same time, in spite of its thinness, the cuticle in many microinsects remains divided into the epi-, exo-, and endocuticle (in some, only the division into the epi- and procuticle is observable) (64–66). The hypoderm of microinsects forms an uneven layer, in some areas so thin that it is barely discernible (66).

Reduction of body size in the studied insects is accompanied by reduction in the number of fully developed elements of the exoskeleton, simplification of the endoskeleton, and reduction in the number of elements in some segmented structures (4, 33, 63–65, 66, 71, 72, 80, 92).

The reduced number of exoskeleton elements is especially pronounced in the heads of beetles: In adult and larval Ptiliidae and Corylophidae, head capsules are whole, without sutures (32, 71, 72). The tentoria of microinsects are simplified to different degrees. The dorsal tentorial arms are absent in many tiny insects. In an extreme case, in Corylophidae (Coleoptera), only the posterior arms and tentorial bridges are present (72). The larvae of Trichogrammatidae and Mymaridae have no tentoria (41). The structure of the mouthparts in the majority of microinsects is not markedly simplified, except in the males of *Dicopomorpha echmepterygis*, which have no mouthparts, and in the larvae of Trichogrammatidae and Mymaridae, which have only small or rudimentary mandibles

(1, 6, 42). In spite of the small body size, the number of antennomeres is not strongly reduced in the majority of studied insects. Exceptions are in some genera of Ptiliidae and Corylophidae where the number of antennomeres in the adult is reduced from 11 to 8 (8, 35, 80), and in males of *D. echmepterygis*, which have only one antennomere (55).

The structure of the skeleton of thoracic segments greatly varies in different microinsects. Among adult microinsects, thrips (*Heliothrips haemorrhoidalis*), which have eight differentiated sclerites in the prothorax (54), are at one extreme, and Ptiliidae and Corylophidae, in which the entire exoskeleton of the prothorax is composed of a single functional element, the pronotum with hypomeron (71, 72), are at the other extreme. Generally, the miniaturization of adults is often accompanied by the fusion of pleural elements and less often accompanied by the fusion of dorsal and ventral elements (70–72). The larvae and nymphs of the majority of microinsects have poorly pronounced sclerites (57), no developed sclerites except for the weakly sclerotized notum (larval Ptiliidae and Corylophidae; 71, 72), or no sclerites altogether (late instar larvae of Mymaridae and Trichogrammatidae; 1, 6, 42). The thoracic endoskeletons of the majority of microinsects are greatly flattened, with nonbranching furcae; in *Megaphragma* the metafurcae are absent (69); in *Mengenilla chobauti* (Strepsiptera) the first instar larvae lack any elements of thoracic endoskeleton (61). In the larvae and nymphs of the majority of microinsects, the furcae are even less pronounced than in the adults. One distinguishing feature of Trichogrammatidae is the hypertrophied mesophragma, which is sunk in the body and reaches the middle of the abdomen in *Trichogramma* and almost reaches the apex in *Megaphragma* (69).

The legs of all microinsects are divided into parts typical of insects. The number of tarsomeres is smaller in the majority of microinsects than in larger members of related groups. The tarsi have two or three segments in Ptiliidae (35), three segments in Corylophidae (8), and one or two segments in thrips (54); in the males of *Dicopomorpha* tarsomeres are absent (65). The wing apparatus of the majority of microinsects is characterized by developed ptiloptery and reduced number of veins (65, 70, 71). The wings of all flying microinsects are narrow and contain at most only three veins, often very weakly developed; sometimes the number of veins is reduced to one. The main plane of the narrow wing is formed by long setae running along the perimeter (**Figure 2a**). In Ptiliidae these setae bear numerous processes (**Figure 2b**), which probably serve to increase the total area of the wing (71).
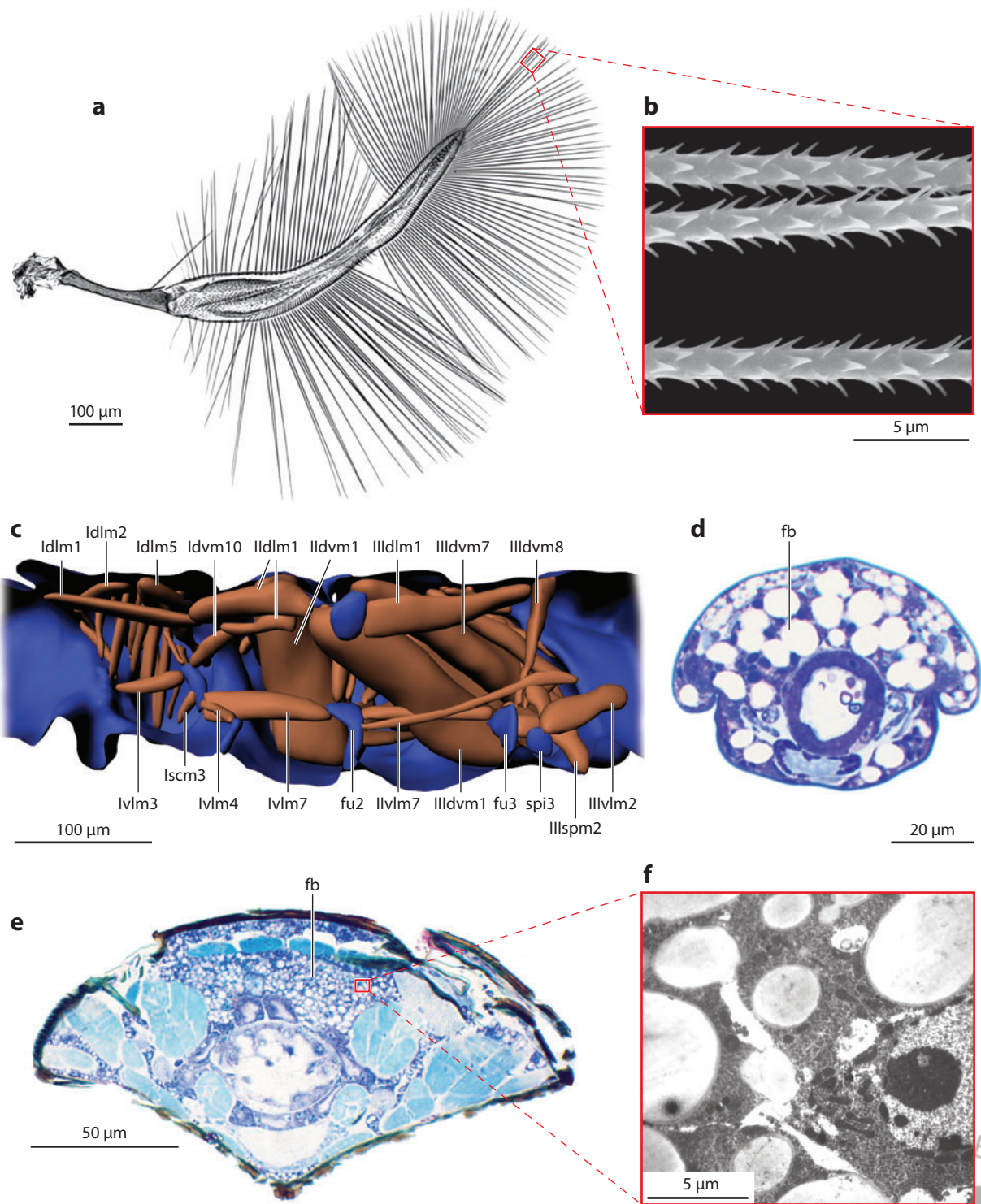
The skeleton of the abdomen in adult microinsects displays two opposite trends: On the one hand, the number of distinguished elements is reduced in some of the smallest hymenopterans, such as *Megaphragma mymaripenne*, which have almost indiscernible sternites (69), or males of *D. echmepterygis*, which have only two abdominal segments (55); on the other hand, ten well-developed segments have been described in Ptiliidae (71) and Thripidae (56). Microinsect larvae lack developed elements of the skeleton in the abdomen (65, 71, 72).

The male copulatory apparatus of Ptiliidae and Corylophidae differs from that of larger members of related taxa in the absence of parameres (71, 72). The ovipositor and male copulatory

Ptiloptery: condition of the wing apparatus characterized by a narrow wing plate surrounded with a fringe of long setae

Figure 2

Peculiar features of microinsect structure: (*a,b*) wing of *Acrotrichis montandoni* (Coleoptera: Ptiliidae); (*c*) three-dimensional reconstruction of thoracic musculature of *Heliothrips haemorrhoidalis* (Thysanoptera: Thripidae), lateral view (*blue*, cuticle; *brown*, muscles); (*d–f*) fat body of Ptiliidae (Coleoptera); (*d*) last-instar larva of *Mikado* sp., cross section of abdomen; (*e,f*) adult *A. montandoni*, cross section of mesothorax. Abbreviations: Idlm1, Musculus prophragma-occipitalis; Idlm2, M. pronoto-occipitalis; Idlm5, M. pronoto-phragmalis anterior; Idvm10, M. profurca-phragmalis; Iscm3, M. profurca-coxalis medialis; Ivlm3, M. profurca-tentorialis; Ivlm4, M. profurca-spinalis; Ivlm7, M. profurca-mesofurcalis; IIdlm1, M. prophragma-mesophragmalis; IIdvm1, M. mesonoto-sternalis; IIvlm7, M. mesofurca-abdominosternalis; IIIdlm1, M. mesophragma-metaphragmalis; IIIdvm1, M. metanoto-sternalis; IIIdvm7, M. metanoto-trochanteralis; IIIdvm8, M. metafurca-phragmalis; IIIspm2, M. metafurca-pleuralis; IIIvlm2, M. metafurca-abdominosternalis; fb, fat body; fu2, 3, meso- and metafurca; spi3, metaspina.

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a

100 μm

b

5 μm

c

Idlm1 Idlm2 Idlm5 Idvm10 IIdlm1 IIdvm1 IIIdlm1 IIIdvm7 IIIdvm8

Iscm3

Ivlm3 Ivlm4 Ivlm7 fu2 IIvlm7 IIIdvm1 fu3 spi3 IIIvlm2

IIIspm2

100 μm

d

fb

20 μm

e

fb

50 μm

f

5 μm

apparatus of the smallest hymenopterans display no substantial changes, except for the absence of parameres in *Megaphragma* (69).

Musculature

Comparison of the musculatures in adult microinsects and members of related taxa reveals only a few reductions not found in larger relatives (70–72). Analysis of the peculiar features of musculature found in different microinsect taxa has shown no reductions common to all microinsects and only three modifications common to several microinsect taxa (71, 72). It can be concluded that the effects of miniaturization on musculature in insects are rather minor (**Figure 2c**).

The structure of the musculature is considerably more conserved than the structure of the skeleton (51), making musculature especially important for functional morphology and taxonomy. The high degree of stability in muscles in spite of dramatic changes in body size makes the musculature-related set of characteristics indispensable for taxonomy, including macrosystematics, because they can be used to compare large taxa that greatly differ in size (70, 93).

One interesting feature of musculature has been found in the adult *Sericoderus lateralis* (Coleoptera, Corylophidae), in which the musculature degenerates in the course of postpupal development as a result of reproductive system maturation (67).

Digestive and Excretory System

The alimentary canals of all microinsects are similar in principal structure and differ from those of larger members of related groups in the somewhat weaker differentiation into sections. The majority of microinsects lack crops and gizzards (64–66). In Ptiliidae and Corylophidae, disappearance of longitudinal muscles of the esophagus has been recorded, and all studied microinsects lack midgut musculature (70–72). The number of Malpighian tubules in the smallest beetles is equal to the minimum number found in larger members of related groups (71, 72); Mymaridae and Trichogrammatidae have fewer Malpighian tubules than the larger Chalcidoidea (65, 69). In many microinsects, salivary glands are absent (65, 66).

Circulatory System and Fat Body

The circulatory systems of the smallest insects are greatly simplified; no vessels or pulsatile organs, except for the simple heart and short aorta, have been found in any of them, and in Ptiliidae and Trichogrammatidae the heart and vessels are absent (64, 65, 71). In Ptiliidae the hemolymph is supplanted by parenchyma-like fat body (**Figure 2d–f**) (64). Similar organization is found in many acari, especially small ones (83).

Tracheal System

The structure of the tracheal system becomes considerably simplified with decreasing body size. In the majority of microinsects, it is represented only by longitudinal stems and a few branching tracheae (70). The number of spiracles is smaller than in larger insects; in some cases, only one pair of spiracles is present (larval Ptiliidae; 33, 71) or spiracles are absent (larval Trichogrammatidae and Mymaridae; 6). The larvae of Trichogrammatidae and Mymaridae develop in the host egg, have no tracheal system, and breathe through their integument (41). The larvae of Ptiliidae are free-living but have only one pair of spiracles (mesothoracic), lack tracheal stems and trachea in the abdomen, and probably employ mixed tracheal-cutaneous respiration (64).

Central Nervous System

All microinsects display strong oligomerization and concentration of ganglia (64–66, 71, 72). The highest degree of compaction and concentration of the nervous system among microinsects is found in beetles. The central nervous systems of Corylophidae are represented by a single formation with poorly discernible ganglia (**Figure 3a**). Another unique feature of this family is the complete shift of the head ganglia into the thorax at the adult stage (72). The central nervous systems of the larvae of tiny beetles display considerably less ganglion convergence and fusion, but their brains are always situated outside the head capsule; in first instar larvae of *Mikado* the brain reaches the second abdominal segment (71). By contrast, thrips and psocopteran nymphs display considerably more ganglion oligomerization and concentration than they do as adults, but their brains always remain in their heads (70). Larval Trichogrammatidae and Mymaridae have only rudimentary central nervous systems (41).

Another important peculiar feature of the central nervous system of microinsects is asymmetry (**Figure 3b,c**). The adults of the smallest Ptiliidae (Nanosellini) have asymmetrical distal processes of the protocerebrum (the left process is larger than the right process) (66). In adult Mymaridae females, the abdominal ganglion shifts into the right half of the abdomen (65). The brain of first instar beetle larvae is strongly asymmetrical, with the left half considerably larger than the right half (71, 72). The subesophageal ganglion and thoracic ganglia of first instar *H. haemorrhoidalis* larvae have asymmetrical processes protruding into the coxal areas of the legs (70). The shift of part of the subesophageal ganglion into the coxa of the legs has also been described in the nymphs of small spiders (73).

In spite of small body size, the brain in all studied microinsects is completely differentiated into all brain centers typical of larger insects (46, 47). The brains of the smallest insects display strong compaction and convergence of brain centers, as well as changes in the spatial organization of these centers. Their brains not only retain the complete set of brain centers but also almost retain the proportions of particular parts (46, 47). The neuropil areas of each brain center change strictly isometrically (e.g., in the central body complex in beetles) or display minor allometry. One common trend of almost all microinsect taxa is the slight decrease in the relative volume of areas performing the functions of coordination and processing of sensory signals (optic and antennal lobes) (46, 47).
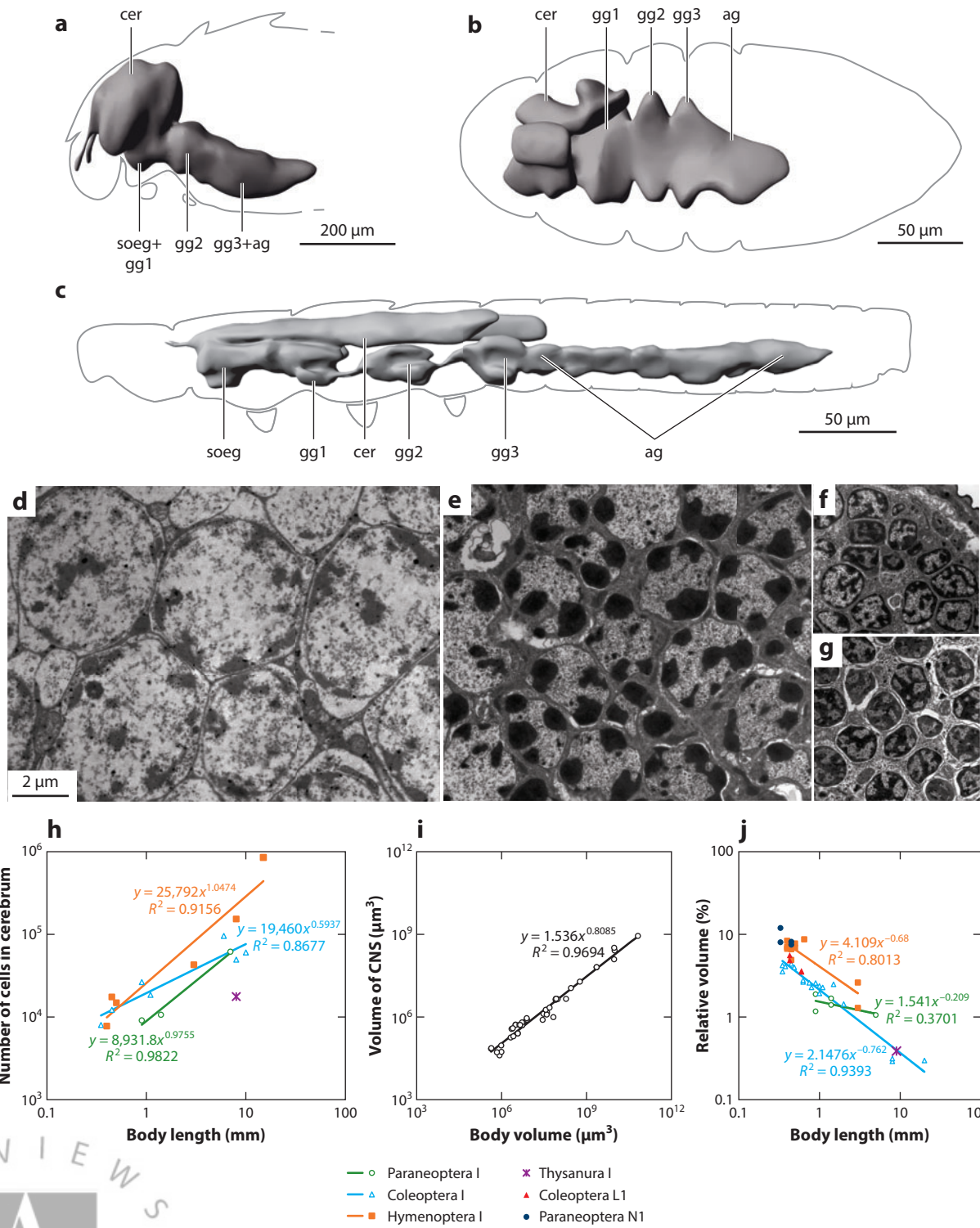
Decrease in body size is accompanied in all studied insects by a considerable decrease in the size of cells in the nervous system (**Figure 3d–g**). This trend can be traced in all studied orders (4, 5, 46, 47, 64, 65). The average neuron size of the smallest insects is about 2 μm (46, 47). This decrease in size is accompanied by lower variation in the size of the neurons: Larger insects of the same species can have cell body size varying by a factor of more than 10, whereas in the smallest insects this variation is at most by a factor of 2.5 (46). The decrease in neuron size in the smallest insects is accompanied by considerable changes in the nuclear-cytoplasmic ratio: The volume of the cytoplasm decreases, and the nucleus occupies up to 90% of the cell volume (64). In the smallest Ptiliidae, Mymaridae, and Trichogrammatidae, considerable increase of chromatin compaction has been recorded (47, 64–66). In spite of the decreased neuron size and cytoplasm volume in cell bodies and processes, the studied microinsects retain complete sets of organelles typical of neurons (46, 47). The set and structure of the membranes and glia of the central nervous system of microinsects also show no differences from those of larger insects (46, 47). Microinsects also have considerably fewer neurons than larger members of related taxa (46, 47, 66). The smallest insects have only about 10,000 cells in the brain (**Figure 3h**).

A unique structure of the nervous system has been found in one of the smallest members of the family Trichogrammatidae, *M. mymaripenne* (68). The central nervous system of the pupa in this

Oligomerization: decrease in the number of homologous or metameric organs through merger or reduction (see Reference 17)

a cer

soeg+ gg2 gg3+ag
gg1

200 μm

b cer gg1 gg2 gg3 ag

50 μm

c

soeg   gg1   cer gg2   gg3   ag

50 μm

d

2 μm

e

f

g

h

$y = 25{,}792x^{1.0474}$
$R^2 = 0.9156$

$y = 19{,}460x^{0.5937}$
$R^2 = 0.8677$

$y = 8{,}931.8x^{0.9755}$
$R^2 = 0.9822$

Number of cells in cerebrum

Body length (mm)

i

$y = 1.536x^{0.8085}$
$R^2 = 0.9694$

Volume of CNS (μm³)

Body volume (μm³)

j

$y = 4.109x^{-0.68}$
$R^2 = 0.8013$

$y = 1.541x^{-0.209}$
$R^2 = 0.3701$

$y = 2.1476x^{-0.762}$
$R^2 = 0.9393$

Relative volume (%)

Body length (mm)

— ○ Paraneoptera I   ✖ Thysanura I
— △ Coleoptera I   ▲ Coleoptera L1
— ■ Hymenoptera I   ● Paraneoptera N1

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species contains 7,400 nuclei and has a structure typical of insects, with each ganglion consisting of neuropil surrounded by a cortical layer of cell bodies of neurons. The entire pupal central nervous system occupies 19% of the body volume; the supraesophageal ganglion contains 4,600 cells and occupies 11% of the body volume. At the final stages of pupal development, the nuclei and cell bodies of more than 95% of the neurons undergo lysis, prior to the emergence of the adult from the pupa. As a result, the adult central nervous system contains only 360 nuclei, 215 of them in the supraesophageal ganglion. Given that the volume and structure of the neuropil are almost identical in the adults and pupae of *M. mymaripenne*, the numbers of neurons in the adults and pupae are presumably also identical.

Sense Organs

Decrease in body size in insects is accompanied by a considerable decrease in the number of sensilla. This decrease is especially visible in the antennae. In *M. mymaripenne* each antenna bears only 38 sensilla, whereas in the other Chalcidoidea each antenna bears more than 1,000 sensilla (70). The size of sensilla also decreases with decreasing body size, although the scale of these changes is smaller than the scale of the changes in number (70). In *Megaphragma* the smallest sensilla are at most 0.6 μm in diameter; in the other Chalcidoidea sensillum diameter is from 0.8 to 12 μm (69). In the smallest beetles, the numbers of sensilla are also greatly decreased, compared with larger members of related taxa, and the smallest basal diameter of sensilla found in Ptiliidae is 0.65 μm; in large related Staphylinoidea the diameter is not less than 1 μm (70).

Decrease in body size is also accompanied by a dramatic decrease in the number of ommatidia in the eyes (25, 26): In the smallest insects, each eye contains only 30 ommatidia (48, 70). Comparison of the smallest Chalcidoidea (*Megaphragma*) with medium-sized ones shows a difference in the number of ommatidia by a factor of almost 60 (70). At the same time, these insects differ in the diameter of one ommatidium only by a factor of 2. The smallest known ommatidium in insects is 6 μm in diameter (*Trichogramma evanescence*, 26); in the other Chalcidoidea the diameter is from 8 to 13 μm (70).

Reproductive System

The structure of the reproductive system of the majority of microinsects shows little difference from that of larger insects: It includes paired gonads, all glands typical of related taxa (even in the smallest hymenopterans, 69), copulatory apparatus, and ovipositor. But in some of the smallest insects the reproductive system is asymmetrical, with one gonad considerably larger than the other (65) or, as in male and female Ptiliidae, with only one gonad present (64, 66, 71). The number of ovarioles in microinsects is decreased, and the smallest of them have only two ovarioles in each ovary (*Megaphragma*, 69) or in the only ovary (Nanosellini, 71). Decreased body size is

Figure 3

Effects of miniaturization on the insect nervous system. (*a,b*) CNS of *Sericoderus lateralis* (Coleoptera: Corylophidae) (modified from 72): (*a*) adult, lateral view; (*b*) first instar larva, dorsal view. (*c*) CNS of first instar larva of *Mikado* spp. (Coleoptera: Ptiliidae), lateral view (modified from 71). (*d–g*) Neurons of beetles of different sizes (Coleoptera), transmission electron micrographs: (*d*) *Aleochara bilineata* (Staphylinidae), (*e*) *Acrotrichis grandicollis*, (*f*) *Mikado* sp., (*g*) *Nanosella* sp. (Ptiliidae). (*h*) Changes in the number of cells in the brain in different taxa. (*i*) Changes in CNS volume. (*j*) Changes in relative brain volume in different taxa. Data from References 46, 47, 66, 70–72. Abbreviations: ag, abdominal ganglia; cer, cerebrum; CNS, central nervous system; gg1–3, pro-, meso-, and metathoracic ganglia; soeg, subesophageal ganglion.

accompanied by the disproportionately larger size of spermatozoa: In some species of Ptiliidae spermatozoa are even longer than the body (15, 20).

COMPARISON OF MINIATURIZATION EFFECTS IN DIFFERENT INSECT TAXA

Comparison of morphological changes related to decreased body size shows that the majority of these changes are convergent and evolved independently in different insect taxa.

Characteristics of the Majority of Microinsects

All or the majority of microinsects have the following characteristics (most of them are reductions or simplifications) (70):

- reduction of sutures and fusions of sclerites in the head skeleton of adults and larvae,
- simplification of the tentorium in adults and larvae,
- fusion of thoracic sclerites in adults,
- absence of most sclerites and extremely weak sclerotization of the integument in larvae,
- simplification of the endoskeleton of thoracic segments,
- ptiloptery,
- reduction in the number of wing veins,
- significantly flattened hypoderm cells,
- simplification of alimentary canal differentiation,
- reduction of gut musculature,
- reduction in number of Malpighian tubules,
- simplification of the circulatory system,
- simplification of the tracheal system in adults and larvae (reduction in number of spiracles),
- oligomerization and concentration of the central nervous system,
- asymmetry of the central nervous system,
- increase in nuclear-cytoplasmic ratio of neurons,
- considerable reduction in size and number of neurons,
- increase in relative volume of the central nervous system and brain,
- dramatic reduction in number of ommatidia and sensilla,
- reduction in number of ovarioles, and
- increase in the relative volume of the reproductive system.

Characteristics of Some Microinsects

Some microinsects have the following characteristics (70):

- reductions in numbers of antennomeres, palpomeres, and tarsomeres (Ptiliidae, Mymaridae, and Trichogrammatidae);
- reduction in number of tergites and sternites (adult Mymaridae and Trichogrammatidae);
- simplification of the male copulatory apparatus (Ptiliidae and Corylophidae);
- absence of cuticle differentiation into exo- and endocuticle (Mymaridae and Trichogrammatidae);
- reduction of the tracheal system and an accompanying shift to partly or entirely cutaneous respiration in larvae (Ptiliidae, Mymaridae, and Trichogrammatidae);
- absence of a heart (adult and larval Ptiliidae, adult and larval Trichogrammatidae, and larval Mymaridae);

- shift of the abdominal ganglia into the thorax (Liposcelididae, Ptiliidae, and Corylophidae);
- considerable increase in the degree of chromatin compaction in nuclei of neurons (Ptiliidae, Trichogrammatidae, and Mymaridae);
- reduction of particular muscles; and
- desembryonization and strong simplification of structure in larvae (Mymaridae and Trichogrammatidae).

Unique Features of Particular Microinsects

Each of the following characteristics is known to occur in only one family of microinsects:

- absence of all elements of the thoracic endoskeleton (first instar larva of *M. chobauti*; 61);
- mechanism of folding, fixation, and protection of wing (Ptiliidae; 70);
- replacement of hemolymph with parenchyma-like fat body (Ptiliidae; 64, 66, 71);
- complete shift of the brain into the thorax in adults (Corylophidae; 72);
- lysis of cell bodies and nuclei of neurons (*Megaphragma*; 68);
- lysis of wing musculature during reproductive system maturation (*Sericoderus*; 67);
- unpaired gonads (Ptiliidae; 64, 66, 71); and
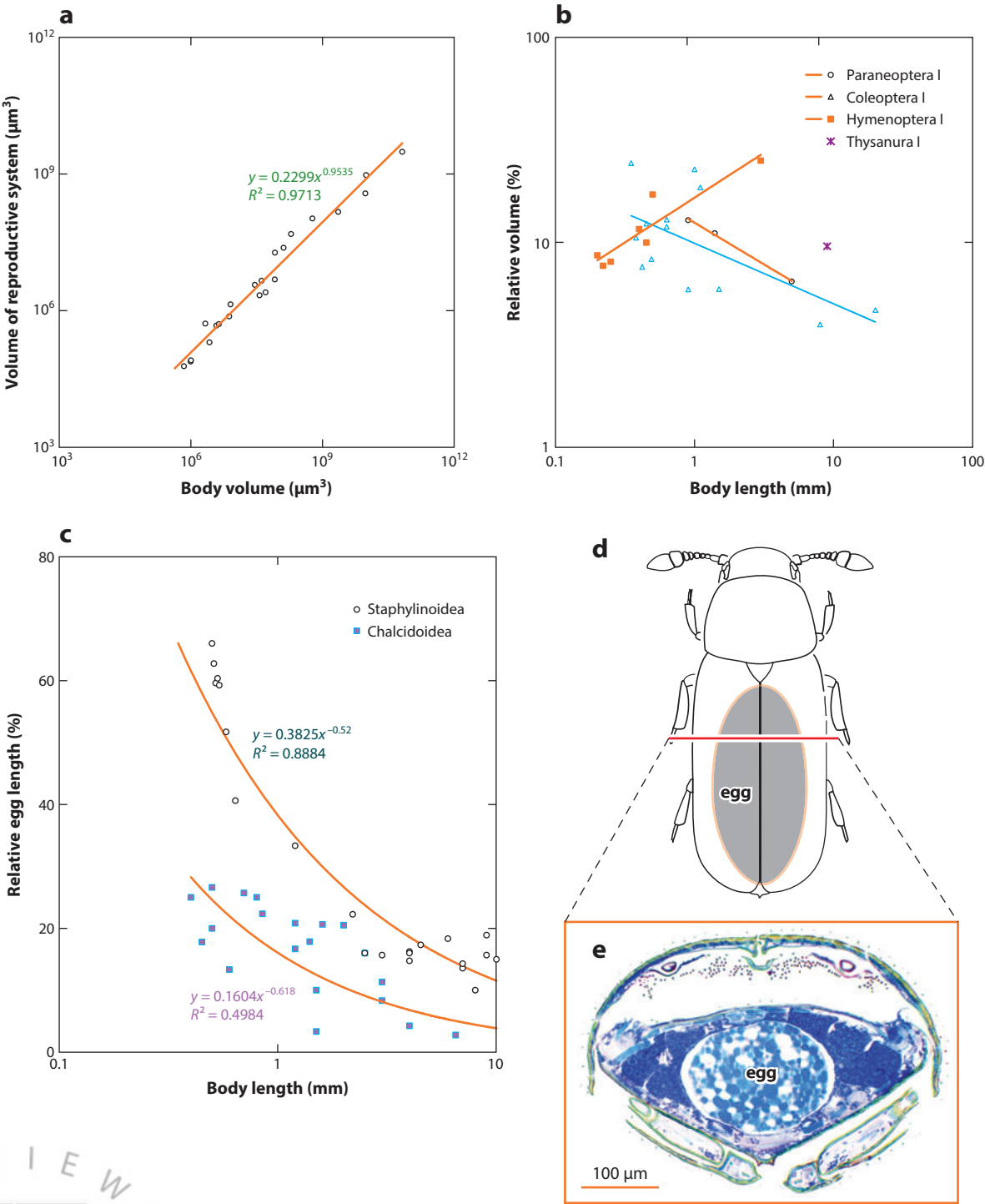- critical simplification of structure in males (*Dicopomorpha*; 38, 65).

The smallest insects and all insects less than 0.4 mm long are holometabolous. The relatively larger size of the smallest hemimetabolous insects can be explained by the practically identical body plan of the nymphs and adults. In this case, the first instar nymph, rather than the adult, should be viewed as the main limiting stage: On the one hand, it is the size of the first instar nymph that limits the size of the egg, and on the other hand, the first instar nymph has almost the same structure as the adult. The possibility of stepwise oligomerization in Holometabola (17) gives them greater opportunities for miniaturization compared with Hemimetabola.

All insects with body length smaller than 0.3 mm are wasp parasitoids of eggs. This is largely determined by the considerably simplified structure of their larvae; strong desembryonization; and reduction of yolk in the egg, which in some species is entirely alecithal: These features are possible in larvae that develop inside host eggs (41).

LIMITS TO INSECT MINIATURIZATION

One of the earliest authors who started the discussion of factors that limit the minimum size of animals was Bernhard Rensch. He proposed that the size of animals is limited by the size of the egg, which is disproportionately large in many small animals; the smallest invertebrates (e.g., Gastrotricha, Rotatoria, and the smaller mollusks) have only one egg developing at a time, and the smaller amphibians and reptiles have at most two eggs developing at a time (74). The second idea proposed by Rensch is that the size of animals is limited by the maximum filling of the body with organs. This idea was based on the allometry of wing musculature in small dipterans and aphids and on the observation that the brain of the smaller insects occupies almost the entire head capsule (74). Studies of relative volumes of organs in the smallest insects and larger members of related taxa have shown that (*a*) metabolic systems, circulatory systems, and musculature change isometrically or display decreasing relative volume with decreasing body size (in the majority of insect taxa), with relative volume of the skeleton slightly increasing, and (*b*) only the relative volumes of the central nervous system (in all insects) and reproductive system (in most) increase by a considerable factor with decreasing body size (**Figures 3i,j** and **4a,b**); hence, it is through analysis of these two systems that the factors limiting the minimum size of insects can be identified (64, 66, 70, 71).

a

$y = 0.2299x^{0.9535}$
$R^2 = 0.9713$

b

- ○ Paraneoptera I
- △ Coleoptera I
- ■ Hymenoptera I
- ✳ Thysanura I

c

- ○ Staphylinoidea
- ■ Chalcidoidea

$y = 0.3825x^{-0.52}$
$R^2 = 0.8884$

$y = 0.1604x^{-0.618}$
$R^2 = 0.4984$

d

egg

e

egg

100 μm

The first hypothesis on the factors limiting the miniaturization of insects concerns the nervous system. The sizes and numbers of neurons in microinsects are considerably smaller than in other insects (46, 47, 66, 68). Nevertheless, the relative size of the central nervous system (**Figure 3i**) in the smallest insects is greater by a considerable factor than that in larger ones (30, 64–66, 70–72, 74). In extreme cases, the relative volume of the central nervous system can reach 11.8% in adults (*T. evanescence*) and 16.75% in first instar larvae (*Liposcelis* sp.) (70). Increasing relative size of the central nervous system with decreasing body size has also been found in other animals (73). The relative brain size, or index of encephalization, is widely referred to in discussions of the evolution of neural activity in animals (77). The relative brain size in humans is 2.5%. The highest previously known index of encephalization among all animals, 8.33%, was recorded in hummingbirds (Trochilidae). However, in *T. evanescence* this index reaches 8.36%, and in the first instar nymph of *Liposcelis* sp. it reaches 11.95% (47, 70). The principle of allometric changes of brain size with changing body size, known as Haller's rule or brain-body allometry, has been shown to be true of many insects (4, 5, 23, 46–49, 64–66, 74, 75, 78, 89), spiders (73), and other invertebrates (23). The only known exceptions to Haller's rule among all animals are several lineages of laboratory cultures of *Trichogramma* (91). The dramatically increasing relative volume of the central nervous system in the smallest insects not only limits the body construction geometrically (for example, in many insects, brain volume is greater than the volume of the head) but also leads to strongly increasing metabolic expenses determined by the increasing relative volume of the central nervous system and the increasing relative surface area of one neuron, and consequently leads to increasing energy cost (11).

> **Index of encephalization:** ratio between brain mass (or volume) and body mass (or volume)

The minimum size of the nervous system is limited by its conserved structure and ultrastructure and by the number and size of neurons (46, 47). Retaining a considerable number of neurons is required for performing the functions of the central nervous system, because the number of neurons determines the efficiency of the nervous system (43). The nuclear-cytoplasmic ratio for neurons is considerably higher in microinsects than in other insects. In neurons of the smallest insects, the nucleus occupies up to 90% of the cell volume (66). Hence, the size of the neurons in the smallest insects approaches the minimum value limited by the nucleus size. The size of the nucleus, in turn, is limited by the size of the genome (34). This is also evidenced by the fact that the size of nuclei in the smallest insects decreases via the decreasing degree of chromatin compaction (66). The second factor limiting the miniaturization of the nervous system is the diameter of the processes of neurons. According to mathematical models proposed by Faisal et al. (24), decreasing diameter of the processes increases the noise effects of ion channels and, as a result, disturbs the conduction of action potentials. Judging by these models, axons smaller than 80 nm in diameter are incapable of conducting the signal (24). Processes smaller than this theoretical limit have been found to occur sporadically in the smallest insects (40), but the majority of their axons are wider, and therefore the limits implied by this model actually apply to the size of neurons. Another consequence of these limits is that in spite of the obvious difficulties in limiting the cell body size of neurons (related to the size of the nucleus and principal organelles), the relative volume of the neuropil in all studied insects is practically constant (the average value for all studied insects is 59.4%) and changes isometrically to the brain size with decreasing body size (46, 47).

Figure 4

Effects of miniaturization on the reproductive system. (*a*) Changes in volume of the reproductive system. (*b*) Changes in relative volume of reproductive systems in different taxa. Data for panels *a* and *b* from References 66, 70–72. (*c*) Changes in relative egg size. Data from various sources cited in Reference 70. (*d,e*) Egg of *Porophilla mystacea* (Coleoptera: Ptiliidae).
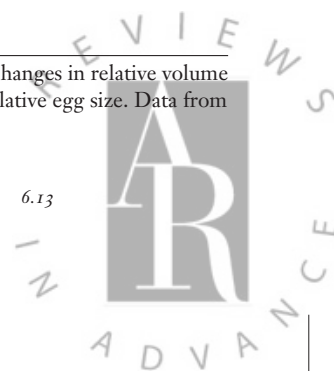
In the studied hymenopterans, the central nervous system clearly limits the size of the adult, because in the larvae it is only rudimentary (41, 70). In the beetles, both the first instar larva and adult are important, because the former has the highest relative volume of the central nervous system during postnatal development (71, 72) and the latter has considerably more complex organization and a greater number of neurons (46). In hemimetabolous insects, the critical stage is the first instar nymph, which has a considerably greater relative volume of the central nervous system than at the other stages (3.8 times as great as in the adult in *Liposcelis* sp. and 4.7 times as great as in *H. haemorrhoidalis*); central nervous system and brain differentiation in the first instar nymph and in the adult are almost indistinguishable (70).

The nervous systems of animals smaller than or comparable in size to the smallest insects are principally simpler and consist of two or three hundred neurons (50, 53, 90). Therefore, the size of these animals is less strongly limited by the central nervous system.

The second hypothesis concerns the size of the egg and the volume of the reproductive system. It is based on my analysis of published data on egg size in Staphylinoidea and Chalcidoidea (for the list of sources, see 70). In Coleoptera, by the example of Staphylinoidea, the relative egg size increases by a factor of more than six with decreasing body size (**Figure 4c**). In Ptiliidae, only one egg develops at a time (**Figure 4d,e**), occupying more than half the body length of the female (19). It is probably the minimum size of the egg that limits the further miniaturization of beetles. The need to develop such an egg explains the strong increase in the relative volume of the reproductive system. In Chalcidoidea the relative egg size increases much less (**Figure 4c**), because the larva develops in the egg of the host, making possible the strong desembryonization and depletion of yolk, up to completely alecithal eggs (41). This also explains the relatively small volume of the reproductive systems found in Mymaridae and Trichogrammatidae. Increasing relative egg size with decreasing body size has also been recorded in other insects (27, 28). Therefore, Rensch's idea that the minimum size of animals is limited by the size of the egg (74) is true in insects, except for those hymenopterans that have greatly simplified parasitic larvae.

The size of the sense organs should also be considered among the factors that limit the minimum size of insects. One of the effects of miniaturization on sense organs is the dramatic reduction in the number of structural units (sensilla, ommatidia), but the size of particular sensilla and ommatidia changes only slightly (48, 70). The insect sensillum is a complex receptor structure that consists of a cuticular part and several cells (79). This construction is apparently sensitive to scaling and limits the further diminution of the sensillum below a minimum diameter of 0.6 μm. The limit to the miniaturization of the ommatidium is about 6 μm in diameter (48). This limit is determined by several factors. One of them is structural. Even in one of the smallest insects, such as *M. mymaripenne*, each ommatidium consists of 21 cells (48). All these cells have nuclei. Although almost all chromatin in these cells is compacted, the diameter of the majority of their nuclei is more than 1 μm. The nucleus size clearly precludes further diminution of the ommatidium. The second factor limiting the ommatidium size is optical. The optical efficiency of the ommatidium is determined by the size of the lens and the area of the rhabdom (44, 87, 88); as a result, these two parameters cannot be decreased ad infinitum. The length of the rhabdom decreases considerably with decreasing body size (48). It is probably to retain the focal power of the ommatidium that the rhabdom diameter and lens size in *M. mymaripenne* are greater than in the larger *T. evanescence* (48). It has also been shown that small insects approach the diffraction limit of vision (26). In addition to constructive limits, there is probably an energy limit to the size of receptors, because relative energy expenses increase with decreasing size (58, 59).

The last possible hypothesis concerns feeding and the need to overcome the surface tension force and capillary forces. This is the decisive factor limiting the minimum size of sucking insects (60). It is probably also quite important for other insects.

CONCLUSIONS

Gorodkov (31) set the threshold of miniaturization (pumilization) at about 1 mm and suggested that overcoming this limit should be accompanied by dramatic simplification, which he termed pumilistic degeneration. It should be noted that this is true of many invertebrate taxa (76, 82). But microinsects go below this threshold without much simplification, and even the smallest of them not only are free of any considerable signs of simplification but also even display a number of novel structures. Thus, insects demonstrate an amazing ability to scale biological structures and processes: The smallest insects differ from other animals of comparable size in their principally higher levels of structural organization and considerably greater numbers of cells (70).

Considerable structural changes of microinsects related to miniaturization are associated with renunciation of certain physiological functions (lack of wings, legs, mouthparts, sense organs) or considerable changes in proportional effects of physical forces and relative environmental parameters, such as capillary forces, air viscosity, or diffusion rate (reduction of the tracheal and circulatory systems, ptiloptery). The nervous and reproductive systems are especially intolerant of miniaturization. It is by these systems that the minimum body size of insects is limited.

Decreasing body size can lead to considerable physiological reorganization, as repeatedly shown for vertebrates, although data on insects are almost absent (36, 77). The data on the association between the complexity of behavior and brain size are of special interest (3), but for insects such data are available only for social species (13, 23). The behavior of microinsects has never been specifically studied, but all principal behaviors displayed by larger members of related taxa can also be found in microinsects, such as tiny hymenopterans (9, 62). For microscopic spiders decreasing body size does not lead to simplification of behavior (21, 22).

The described peculiar morphological features related to miniaturization and the patterns of the scaling of organs in insects open a number of new fields in the study of the effects of miniaturization on animal physiology and ecology and can be used in the search for new biotechnological solutions.

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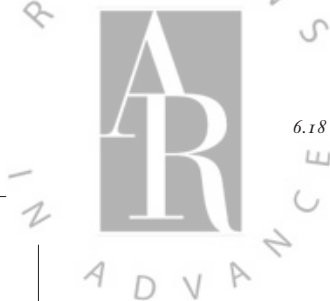
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