Daphnia as a versatile model system in ecology and evolution

Dieter Ebert

Abstract

Water fleas of the genus Daphnia have been a model system for hundreds of years and is among the best studied ecological model organisms to date. Daphnia are planktonic crustaceans with a cyclic parthenogenetic life-cycle. They have a nearly worldwide distribution, inhabiting standing fresh- and brackish water bodies, from small temporary pools to large lakes. Their predominantly asexual reproduction allows for the study of phenotypes excluding genetic variation, enabling us to separate genetic from non-genetic effects. Daphnia are often used in studies related to ecotoxicology, predator-induced defence, host–parasite interactions, phenotypic plasticity and, increasingly, in evolutionary genomics. The most commonly studied species are Daphnia magna and D. pulex, for which a rapidly increasing number of genetic and genomic tools are available. Here, I review current research topics, where the Daphnia model system plays a critical role.

Keywords: Daphnia magna, Daphnia pulex, Cladocera, Branchiopoda, Cyclic parthenogenesis

Natural habitat and life cycle

Daphnia is a genus of small planktonic crustaceans with a very wide geographic distribution. Its English name “water flea,” includes other members of the Cladocera order within the class Branchiopoda, united by the morphology of their trunk limbs (Fig. 1). There are over 100 described Daphnia species, each having a rather similar body architecture characterized by a relatively large head with one simple compound eye and a body encased in a bivalve-like shell (Fig. 2). Daphnia are more or less transparent so as to evade visually hunting predators, e.g., planktivorous fish. They moult four to six times before reaching maturity, but continue to moult and grow in regular intervals throughout their life. Newborn Daphnia (Fig. 2) resemble adults, except that they lack the well-developed dorsal brood pouch of adult females or the secondary sexual traits of males (Fig. 3) [1].

Daphnia are often found in standing freshwater, from very small pools to very large lakes (Fig. 4), and although they may colonize salt water lakes or estuaries, they do not typically colonize sea water, as some species of related genera do. Daphnia are often a keystone species in ponds and lakes, where they are the main primary consumer, filter-feeding small suspended particles, in particular unicellular algae. As such, they play an important role in aquatic food webs, being themselves prey for fish or diverse invertebrate predators.

Daphnia are well known for their ability to reproduce asexually (amictic parthenogenesis, i.e., diploid eggs capable of developing without fertilization) and under favourable conditions they can propagate asexually for many years (Fig. 5). In unstable environments and when environmental conditions deteriorate, Daphnia are able to switch to sexual reproduction. In this case populations produce first males (asexually!) and then haploid eggs that need fertilization. Production of sons and haploid eggs is regulated on the population level, with some genotypes never producing males (so called non-male-producers = NMP) or haploid eggs [2, 3]. Fertilized eggs start development, but then undergo developmental...
Resting embryos require a dormant period before they continue development, resulting in only female hatchlings (Fig. 5). The resting stage, also called ephippium (Fig. 3d), refers to the entire resting structure, i.e., the shell and the resting embryos they contain. Because the embryo is still encased in an egg shell and has an egg shape (Fig. 3e), it is often called resting egg, but this can be misleading. Some lines of Daphnia are known that are able to produce resting stages entirely asexually [4].

In the textbook description of the Daphnia life cycle, sexually produced offspring hatch from resting stages in the spring. These offspring then reproduce asexually across the season and finally terminate the planktonic phase by producing resting stages that endure the harsh winter conditions. However, studies in different habitats have revealed that this is the yearly life-cycle in temperate waterbodies common to Central Europe, where this model system was first developed. Depending on the species and habitat, any ecological aspect of this life-cycle may differ (Fig. 4) [5, 6]. For example, Daphnia may continue in the planktonic form throughout the winter or may persist in short-lived desert rain pools or tiny rock pools. Populations may only be active in winter (rainy season), produce no resting stages or produce resting stages asexually. The commonality among all Daphnia is that asexually reproducing animals are found in habitats, where water temperatures are at least for part of the planktonic season between about 10–30 °C and that they are able to outlive harsh conditions, such as temporary dryness, freezing and periods of low survival probability (e.g., predation, parasitism, toxic water conditions, such
as anoxia) in the form of resting stages. Resting stages can survive many years in pond sediment (and laboratory fridges), as they tolerate drying and freezing.

**Field collections and laboratory culture**

*Daphnia* can be collected from their natural habitats as either planktonic animals or in the form of their resting stages, which are often found in the sediments. Planktonic females can be cloned naturally, enabling researchers to produce unlimited numbers of clonal offspring and keep genetic lines for many generations under laboratory conditions. Clonal lines are kept in either natural fresh water, commercial mineral water or artificial medium (e.g., [7]) and are mostly fed unicellular green algae. On a commercial scale *Daphnia* are even farmed as fish food. Fifteen to 22 degrees is a good water temperature for asexual propagation for most species. Resting stages collected from pond and lake sediments or isolated from sliced and dated sediment cores can be hatched by exposure to oxygen-rich water under daylight and in ambient temperatures. Hatching success is, however, often low, especially for old resting stages, for reasons that we do not yet fully understand, although genetic effects (detrimental mutations) certainly play a role [8]. The oldest
hatched resting stages was about 700 years old [9]. Each hatchling of sexually produced resting eggs is genetically unique and will start to reproduce clonally. Two hatchlings from one resting stage may be half or full-sibs [10].

Clonal reproduction allows us to produce genetically identical replicates so as to entirely control for genetic background. Sexual reproduction in the lab is more time-consuming, because animals (usually small crowded populations) must first be induced to produce male offspring asexually, after which they produce haploid eggs for the males to fertilize [11]. The resulting eggs (actually developmentally arrested embryos) need an obligatory diapause of weeks to months, although methods have been developed to break diapause earlier [12]. Strong differences exist between species regarding the ease of sexual crosses in the laboratory, with *D. magna* currently being the most often-used species for routine crosses. It is possible to cross females with males from the same clone, resulting in genetically selfed offspring [11, 13]. This combination of sexual reproduction [outcrossing and genetic selfing (mother with clonal sons)] and clonal propagation allows researchers to conduct powerful genetic studies and create genetic panels that can be kept by clonal reproduction for many years [13, 14].

**Major interests and research questions**

**Early beginnings**

*Daphnia* research goes back several hundred years, with many important findings dating back to this old model system. To name just a few: August Weismann established his germ plasm theory working with water fleas [15], and Élie Metchnikoff [16] studied macrophages in *Daphnia* (Nobel prize 1908). The concept of phenotypic plasticity was also developed in relation to predator-induced defence in *Daphnia* [17], and the differentiation between prokaryotes and eukaryotes resulted from research on a *Daphnia* and its symbionts [18]. When advancements in modern biology began to be primarily driven by genetic research, *Daphnia* fell somewhat out of favour because of the difficulties in doing genetic crosses—a problem that has only recently been solved. Still, *Daphnia* has remained the prime model in
ecological research and *Daphnia* is one of the best-studied organisms with regard to their ecology, with past or current focal areas are diet vertical migration [19], resurrection ecology [20], host—parasite interactions [21], community ecology [22] and climate change ecology [6, 23]. Moreover, in the last 20 years as genomics and genetics become incorporated into the tool box of *Daphnia* research (primarily *D. magna* and *D. pulex*), this model system is rising in prominence in the field of environmental genomics.

**Phylogenetic position**

*Daphnia* are part of the Branchiopoda, the same group in which the tadpole shrimp *Triops* (Notostraca) and the brine shrimp *Artemia* (Anostraca) are placed (Fig. 1). Since the Crustacea are believed to a paraphyletic group (insects are phylogenetically part of the crustacea but typically not considered crustacea), the Branchiopoda are closer to insects than to other typical crustaceans, such as copepods, Malacostraca, Cirripedia and ostracods. Thus, *Daphnia* may be closer related to *Drosophila* than to lobster. Recent phylogenomic work has placed the genus *Daphnia* firmly in this phylogenetic tree and has clarified the evolution of the Cladocera [24] and the family of Daphniidae [25]. Crustacea are part of the Ecdysozoa, characterized by the need to moult their exoskeleton while growing (Fig. 1).

**Genome**

The *Daphnia pulex* genome was the first crustacean genome to be sequenced [26, 27], followed by several other species [25, 28, 29]. Using flow cytometry, the genome size of several *Daphnia* species has been estimated to be about 230 MB, but genome assemblies are currently much smaller, suggesting that about 25% of the genome—likely the centromeric regions—is yet to be discovered. Several genetic maps have been published, allowing contigs to be sorted and oriented into chromosomes [30–32]. *Daphnia* have 10–12 chromosomes, but polyploid species exist as well.

A major surprise from the first genome was the large number of genes, with early counts around 30,000 [26]. However, a later reassessment reduced this number to about 18,500 [28]. As both these studies used house-made unpublished bioinformatics scripts, it is difficult to understand the source of this difference and determine the better estimate, although unpublished data from *D. magna* (P.D. Fields & D. Ebert, unpublished) suggest that the higher estimate is closer to the real number.

**Local adaptation**

With their habitat limited to standing water bodies, *Daphnia* populations are strongly subdivided. Gene flow is usually moderate, allowing populations to evolve rapidly in line with local environmental conditions. Animals collected from numerous waterbodies have had their genotypes preserved by clonal culture and then tested in common garden experiments (i.e., all animals being raised and phenotyped under the same environmental conditions) or reciprocal transplant experiments (i.e., transplantation of animals among environments, to work out the conditions to which animals are best adapted to). When genotypes demonstrate superior performance under the environmental conditions the animals have evolved in, it is considered evidence of local adaptation. *Daphnia* species have been shown to adapt locally to a long and growing list of environmental factors, including heavy metal pollution, predators, high temperature, habitat stability, photoperiod, water salinity, UV light [5, 33–39].

Although most local adaptation studies are based on comparing a few populations with multiple genotypes per population, a novel approach has been to collect single clones from as many populations as possible, instead of collecting multiple genotypes from each of a few populations. This method allows us to map phenotypes on geographic maps to investigate the influence of geography and climatic conditions (see Fig. 6 for an example how the length of the spina maps across Europe). It assumes, however, that a single genotype is representative of the entire population. The *Daphnia magna Diversity Panel* is a standing collection of hundreds of genotypes, each from a different population used for such studies. Indeed, the strong signal of local adaptation observed across these single genotypes suggests that single genotypes are—at least to some degree—representative of entire populations [6, 33, 39].

**Temporal adaptation and resurrection ecology**

The long-lasting resting stages of *Daphnia* provide an even more compelling demonstration of the high evolutionary potential of these populations. While the majority of resting stages usually hatch during favourable conditions for the planktonic phase, some eggs may remain unhatched, though viable. With time, sediment buries these eggs, archiving them in layered sediments of the past. The field of resurrection ecology avails itself of this archive by collecting sediment cores, slicing them into layers and hatching the embryos from resting stages from the different layers [40, 41]. Information about the age of these layers may be obtained using radio isotope analysis. The resurrected *Daphnia* can then be tested in common garden experiments in response to different environmental variables, preferably those for which a record of past dynamics in the waterbody is present. These studies have revealed the rapid adaptive evolution of different
Daphnia species to diverse environmental factors, such as density of predatory fish [42], heavy metal pollution [40], increased density of toxic cyanobacteria [43], and temporal dynamics in eutrophication [20]. One study resurrected, along with D. magna, the bacterial parasite Pasteuria ramosa, testing and confirming the idea that, over time, hosts and parasites coevolve [44]. Once they are combined with genomic methods, resurrection studies will be able to help us understand genetic targets of selection [45], e.g., by pool-sequencing of embryos from resting stages from different layers and studying the change of alleles (SNP-variants) over time.

Host–parasite interaction

In their natural habitat, Daphnia are often infected at high prevalence with various parasites including viruses, bacteria, microsporidia, fungi, nematodes, cestodes and others (Fig. 7) [46, 47]. Many of these parasites can be co-cultured with their hosts, opening the door for experiments that examine host–parasite interactions on the individual and population levels. Compared to many other host–parasite systems, the Daphnia–micro parasite system offers a level of experimental control that is unsurpassed, enabling us to test basic models of parasite ecology and evolution, such as the mass action principle [48], evolution of virulence [49], host and parasite evolution and coevolution [50, 51], the effects of ageing in host–parasite interactions [52, 53], infections with multiple parasites [54, 55] and parasitism in the face of predation [56] and other stressors [57]. An advantage of the Daphnia system that comes handy with regard to parasitism is the transparency of the host. Many infections can be diagnosed from outside, without killing the host (Fig. 7). Metchnikov’s [16] observation of phagocytosis was possible in vivo, by studying the fate of individual parasite cells in the living host.

The ability to use entire populations, furthermore, enables us to tackle questions that require replication on the population level, such as studies in experimental evolution [58] and epidemiology [59, 60]. Finally, the ease with which it is possible to study parasitism in natural populations allows us to compare data from natural population with data from laboratory experiments [51, 61] to understand the life cycles, epidemiology and evolution of several natural parasites of Daphnia [61].

While many parasites (and epibionts) of Daphnia have been used in field and lab-work, only a few have been studied intensively: the bacteria Pasteuria ramosa [62, 63], the microsporidia Hamiltonsporidium tvaerminnensis [64] and Ordospora colligata [65, 66], the yeast Metschnikowia bicuspidata [21], the chitric fungus Caulerya mesnilli [67] and the Daphnia iridovirus DIV-1 [68]. Current research into the Daphnia parasites is focusing on coevolution and genetic epidemiology [51, 62], with new possibilities opening up as the genomes for most of these parasites becomes available. For example, a GWAS

![Fig. 6 Example for geographic variation in a phenotypic trait: spina length at maturity across populations of D. magna (indicated as black dots). Phenotypes were generated in a common garden experiment and superimposed on a map according to the site of origin of the Daphnia clone. Scale on the right is in mm. On the right are examples of D. magna with short and long spina (red arrows). All genotypes are part of the Daphnia magna diversity panel a standing collection of hundreds of genotypes each from a different population.](image-url)
using different genotypes of *P. ramosa*, recently resulted in the first functional annotation of a parasite gene with regard to *Daphnia*–parasite interaction: a collagen-like protein was found to be responsible for the attachment of the bacteria to the host cuticle [69].

Cloning of *Daphnia* genotypes allows separating the effects of nature (genetic effects) and nurture (environmental effects) to a high degree of sophistication. This has been used to map genes involved in phenotypic traits, including resistance to parasites. Strong variation in host resistance has been reported [50, 50] for several parasites of *D. magna*. Studies that combine QTL-F2 panel mapping and GWAS have identified a number of regions and candidate genes in the *D. magna* genome that contribute to this variation, with strong variation in the underlying genetic architecture ranging, from single gene effects (major QTLs) to complex multigene effects (several minor QTLs), as they are typical for quantitative traits [71–73].

**Phenotypic plasticity**

A hallmark of *Daphnia* biology is its phenotypic plasticity, defined as phenotypic variation expressed by the same genotype in response to environmental cues [74, 75]. Here again, the ability to clone genotypes of *Daphnia* has been instrumental in the development and research of this field. The most readily observable phenotypic plastic trait is the switch between asexual and sexual reproduction (Figs. 3, 5). This switch is triggered by deteriorating environmental conditions that lower the survival likelihood of asexual offspring and, therefore, make the production of sexually produced resting stages less costly [76]. A photoreceptor gene has been mapped in the genome of *D. magna* playing a crucial role for the switch from asexual to sexual reproduction [77].

With a well-developed set of gustatory receptors [78], *Daphnia* are able to sense chemical aspects of their environment, so called “infochemicals” or kairomones [79]. They indicate, for example, the presence of planktivorous fish, invertebrate predators or toxic blue–green algae, allowing the animals to respond in specific ways to reduce the threat (Fig. 8). They may change their body shape (e.g., develop helmets, neck–teeth, elongated tail spines), alter their life-history (e.g., size and age at maturity, offspring size) and change behaviour (phototaxis, swimming parameters) [80–82]. Adaptive phenotypic plasticity also drives responses to environmental conditions. For example, *Daphnia* produce fewer and larger
offspring when food is scarce [83]; and animals produce specific haemoglobin variants tailored to the partial oxygen pressure of the water [84].

Ecotoxicology
Without doubt, the field that spurs the most Daphnia publications is ecotoxicology, which examines the potentially toxic effects of chemical compounds on survival and reproduction. Many countries require that chemical compounds be tested with various organisms before they are produced, released or sold. Daphnia is one of these organisms, mostly D. magna, but also D. pulex, or, more rarely, other species. Guidelines by the Organisation for Economic Cooperation and Development (OECD) require tests, such as the Acute immobilisation test (a short-term or acute toxicity test) and the Daphnia magna Reproduction Test [85, 86]. These tests allow us to produce dose–response curves that can be used to determine parameters, such as the LC50 (lethal concentration at which 50 % animals die) and the lowest observed effect concentration (LOEC), parameters that help determine whether a chemical is potentially harmful for the environment and define any follow-up tests needed to understand the risk.

Moreover, the swimming behaviour of water fleas is increasingly used to quantify the effect of toxins, as well as to monitor the Daphnia response to changes in freshwater supplies for human consumption. Swimming speed, turn frequencies, acceleration and other parameters have all been observed to change as water quality changes. Using video tracking systems, these swimming-related parameters can be estimated in real time, and in the case of flow-through water surveillance systems, ensure that appropriate actions are taken. These systems are much faster than even the acute immobilization test and have become increasingly popular for research and applied aspects [87].

A rarely considered problem with using Daphnia in ecotoxicology is the genetic variation among Daphnia genotypes (clones). Different genotypes of the same species may differ strongly in the estimates of toxicity parameters [88]. Given the commercial interest in the production and sale of chemicals (incl. pharmaceuticals), this variation opens the door for biased reporting. So far, attempts to standardize the genotypes used across the world have not yielded a consensus.

Evolutionary developmental biology
The discovery of the germ plasm by August Weismann in water fleas [15] was a major breakthrough at the time, but in the following hundred years Daphnia was not among the main players in the field of developmental biology. Today, D. pulex and D. magna are frequently used in the evo-devo field, often with a focus on comparative aspects [89], for example concerning the role of Hox genes [90–93], neuro development [94–96] and sex determination [97–100]. Along these lines diverse tools have been developed, for example an atlas for the staging of embryos [101] and diverse tools for genetic manipulation (see next section).
Experimental approaches

RNAi, CRISPR, TALEN

An expanding area of Daphnia research addresses the genetic mechanism underlying phenotypes, with the long-term goal of uncovering gene function in an environmental context. Manipulation of gene expression, as well as gene knock-in and knock-out technology are now possible for Daphnia, with microinjection-based RNA interference (RNAi) used routinely [102–104]. Clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) system have been used for gene knock-outs [105, 106], but also for CRISPR/Cas-mediated knock-in via non-homologous end-joining, e.g., for reporter-genes [107]. Likewise, Transcription Activator-Like Effector Nucleases (TALENs) are increasingly used as a versatile genomic manipulation tool in D. magna [108–110]. These methods are primarily carried out via microinjection into the freshly laid asexual eggs of Daphnia, although other avenues such as bacterial feeding are being developed [111]. Mastering microinjection into the asexual eggs of Daphnia is still the main bottleneck for these methods, because the initially very fragile egg membrane breaks easily, but then hardens fast, so that injection is not possible anymore. The time window with the right conditions is short, even so a method has been developed to stretch it (collect eggs immediately after ovulation in ice-cold medium enriched with 80 mM sucrose) [102]. Another limiting factor is the relatively small clutch size of Daphnia, which in the lab is rarely larger than 30, even so in the field D. magna can produce clutches of more than 100 eggs. [112–114]. However, new protocols allowed to move forward [123, 124]. The number of chromosomes for D. magna is worked out to be 10 (N=1) and is 12 for D. pulex

Transcriptomics

With phenotypic plasticity being a hallmark of Daphnia biology, it is an ideal system to study differential gene expression, as different phenotypes of the same genotype can be contrasted. This has been used intensively for diverse questions related to gene expression differences in different environments or treatments, such as responses to different predators, chemicals, environmental toxins, temperature, salinity, heavy metals, resistance to parasites, sex expression [118–121]. A database, dedicated to gene expression studies in Daphnia (http://www.daphnia-stressordb.uni-hamburg.de/dsdbs tart.php) summaries the result of nearly 100 studies published until 2018 [118]. More than 50 % are from D. magna. Only seven studies of the entire data set analyzed whole-transcriptome expression profiles (using RNA-seq). Besides RNA-seq, proteomics is also rapidly developing for Daphnia as a powerful research tool [122].

Karyotyping

Daphnia have rather small and condensed chromosomes, which posed severe difficulties on accurate karyotyping [123, 124]. However, new protocols allowed to move forward [125]. The number of chromosomes for D. magna is worked out to be 10 (N=1) and is 12 for D. pulex

Research community and resources.

The Daphnia community is rather large with a long tradition. A few hundred papers are published every year that feature Daphnia as the main study organism. The Cladocera community normally holds triannual symposia, but this schedule was interrupted due to the Coronavirus pandemic. A younger meeting series are the “Daphnia Genomics Consortium” meetings, which had convened only three times previously.

Genomic resources

The first genome of D. pulex was already sequenced more than 10 years ago [26] and several genomes of other Daphnia species have since then be sequenced [25, 29] (https://www.ncbi.nlm.nih.gov/genome/?term=Daphnia). Genetic maps exist for D. pulex and D. magna [8, 30, 31].
have a collection of clones from different species and populations by collecting females from natural populations and keeping them clonally in the laboratory, a tradition of widely used standard laboratory lines that has not come about in the Daphnia community. Even in the field of ecotoxicology, several different genotypes are used by labs around the world. Some of the bigger Daphnia research groups have collection of clones from different species and population. One of the biggest collections is currently in the Ebert-research group at the University of Basel, with the Daphnia magna Diversity panel (fully sequenced clonal lines from more than 230 populations). The same lab also houses more than 300 F2 clones of a D. magna QTL panel [30] and more than 100 sequenced D. magna clones from one single population (the Swisspond panel) [51].

Acknowledgements
I thank Christian Laforsch for the pictures for Fig. 8. I thank the entire Ebert-lab for helpful comments and suggestions for this manuscript. Suzanne Zweizig and Maridel Fredericksen improved the language of the manuscript.

Author contributions
The author read and approved the final manuscript.

Funding
This work is supported by The Swiss National Science Foundation.

Availability of data and materials
Not applicable.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The author declares that he has no competing interests.

Received: 30 December 2021   Accepted: 20 June 2022

Published online: 08 August 2022

References
1. Ebert D. Ecology, epidemiology and evolution of parasitism in Daphnia. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information: National Center for Biotechnology Information (US) http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books, 2005.
2. Yampolsky LY. Genetic variation in the sexual reproduction rate within a population of a cyclic parthenogen, Daphnia magna. Evolution. 1992;46:833–7. https://doi.org/10.2307/2409651.
3. Galimov Y, Walser B, Haag CR. Frequency and inheritance of normal producing clones in Daphnia magna: evolution towards sex specialization in a cyclical parthenogen? J Evol Biol. 2011;24:1572–83. https://doi.org/10.1111/j.1420-9101.2011.02288.x.
4. Tucker AE, Ackerman MS, Eads BD, Xu S, Lynch M. Population-genomic insights into the evolutionary origin and fate of obligately asexual Daphnia pulex. Proc Natl Acad Sci USA. 2013;110:15740–5. https://doi.org/10.1073/pnas.1313388110.
5. Roulin AC, Rouitu J, Hall MD, Janicke T, Colson I, Haag CR, Ebert D. Local adaptation of sex induction in a facultative sexual crustacean: insights from QTL mapping and natural populations of Daphnia magna. Mol Ecol. 2013;22:3567–79. https://doi.org/10.1111/mec.12308.
6. Seefeldt L, Ebert D. Temperature-versus precipitation-limitation shape local temperature tolerance in a Holartic freshwater crustacean. Proc Biol Sci. 2019. https://doi.org/10.1098/rspb.2019.0929.
7. Klüttgen B, Dülmer U, Engels M, Ratte HT. AdMa, an artificial freshwater for the culture of zooplankton. Water Res. 1994;28:743–6.
8. Rouitu J, Jansen B, Colson I, De Meester L, Ebert D. The first-generation Daphnia magna linkage map. BMC Genomics. 2010. https://doi.org/10.1186/1471-2164-11-508.
9. Frisch D, Morton PK, Chowdhury PR, Culver BW, Colbourne JK, Weider LJ, Jayasinghe PD. A millennia-scale chronicle of evolutionary responses to cultural eutrophication in Daphnia. Ecol Lett. 2014;17:360–8. https://doi.org/10.1111/ele.12237.
10. Duneau D, Altermatt F, Ferdy JB, Ben-Ami F, Ebert D. Estimation of the propensity for sexual selection in a cyclical parthenogenesis. bioRxiv. 2020. https://doi.org/10.1101/2020.02.05.935148.
11. De Meester L. An estimation of the heritability of phototaxis in Daphnia magna Straus. Oecologia. 1989;78:142–4.
12. Retnaningdyah C, Ebert D. Bleach solution requirement for hatching of Daphnia magna resting eggs. J Zool Life Sci. 2016;6:136–41. https://doi.org/10.11594/jzls.06.03.01.
13. Luijckx P, Fienberg H, Duneau D, Ebert D. A matching-allele model explains host resistance to parasites. Curr Biol. 2013;23:1085–8. https://doi.org/10.1016/j.cub.2013.04.064.
14. Metzger CMJ, Luijckx P, Bento G, Mariadassou M, Ebert D. The Red Queen lives: epistasis between linked resistance loci. Evolution. 2016;70:480–7. https://doi.org/10.1111/evol.12854.
15. Weismann A. Die Continuität des Keimplasmas als Grundlage einer Theorie der Vererbung. Jena: Gustav Fischer; 1885.
16. Metchnikoff ME. Über eine Sproßspilzkrankheit der Daphniden. Beitrag zur Lehre der Phagocytosen gegen Krankheitserreger. Virchows Arch Path Anat Physiol. 1884:9:177–93.
17. Woltereck R. Weitere experimentelle Untersuchungen über Artveränderung speziell über das Wesen quantitativer Artunterschiede bei Daphniden. Verh deutsch zool Ges. 1909;19:110–72.
18. Chatton É. Pansporella perplexa amoeobien a spores protéégées parasite des daphnies. Ann des Sci Nat Zool. 1925;8:5–85.
19. Ringelberg J. Der vertikal migration of zoplankton in lakes and oceans. Dordrecht: Springer; 2010.
20. Brede N, Sandrock C, Straile D, Spaak P, Jankowski T, Streit B, Schwenk K. The impact of human-made ecological changes on the genetic architecture of Daphnia species. Proc Natl Acad Sci USA. 2009;106:4758–63. https://doi.org/10.1073/pnas.0807178106.
21. Duffy MA, Ochs JH, Penczykowski RM, Civitello DJ, Klausmeier CA, Hall SR. Ecological context influences epidemic size and parasite-driven evolution. Science. 2012;335:1636–8. https://doi.org/10.1126/science.1215429.
22. Monchamp ME, Enache I, Turko P, Pomati F, Risnoweau G, Spaak P. Sedimentary and egg-bank DNA from 3 European lakes reveal concurrent changes in the composition and diversity of cyanobacterial and Daphnia communities. Hydrobiol. 2017;800:155–72. https://doi.org/10.1007/s10750-017-3247-7.
23. Brans IJ, Jansen B, Vancovebeke J, Tuzun N, Stoks R, De Meester L. The heat is on: genetic adaptation to urbanization mediated by thermal tolerance and body size. Glob Change Biol. 2017;23:5218–27. https://doi.org/10.1111/gcb.13784.
24. Van Damme K, Cornetti L, Fields PD, Ebert D. Whole-genome phylogenetic reconstruction as a powerful tool to reveal homoplasies and ancient rapid radiation in waterflea evolution. Syst Biol. 2021. https://doi.org/10.1093/sysbio/syab094.
25. Cornetti L, Fields PD, Van Damme K, Ebert D. A fossil-calibrated phylogenomic analysis of Daphnia and the Daphniidae. Mol Phylogen Evo. 2019;137:250–62. https://doi.org/10.1016/j.ympev.2019.05.018.

26. Colbourne JK, Pfrender ME, Gilbert D, Thomas WK, Tucker A, Oakley TH, Tokishita S, Aerts A, Arnold CJ, Basu MK, et al. The ecoregressive genome of Daphnia pulex. Science. 2011;331:555–61. https://doi.org/10.1126/science.1197761.

27. Ebert D. A genome for the environment. Science. 2011;331:539–40. https://doi.org/10.1126/science.1202092.

28. Ye ZQ, Xu S, Spitzer K, Asselmel J, Jiang XQ, Ackerman MS, Lopez J, Harker B, Raborn RT, Thomas WK, et al. A new reference genome assembly for the microcrustacean Daphnia pulex. Genomes Genomes Genet. 2017;7:1405–16. https://doi.org/10.13415/1343108638.

29. Lee BY, Choi BS, Kim MS, Park JC, Jeong CB, Han J, Lee JS. The genome genomic analysis of Daphnia pulex genome. Mol Phylogen Evo. 2019;137:250–62. https://doi.org/10.1016/j.ympev.2019.05.018.

30. Routtu J, Hall MD, Albere B, Beisel C, Bergeron RD, Chaturvedi A, Choi B, Dukic M, Berner D, Roesti M, Haag CR, Ebert D. A high-density genetic map reveals variation in recombination rate across the genome of the freshwater water flea Daphnia magna: a potential use for freshwater molecular ecotocology. Aquat Toxicol. 2019;210:69–84. https://doi.org/10.1016/j.aquatox.2019.02.009.

31. Xu S, Ackerman MS, Long HA, Bright L, Spitze K, Ramsdell JS, Thomas WK, Lynch M. Male-specific genetic maps of the microcrustacean Daphnia pulex based on single-sperm whole-genome sequencing. Genetics. 2015;201:31–8. https://doi.org/10.1373/genetics.115.179028.

32. Dukic M, Berner D, Roesti M, Haag CR, Ebert D. A high-density genetic map reveals variation in recombination rate across the genome of Daphnia magna. BMC Genom. 2016. https://doi.org/10.1186/s12863-016-0445-7.

33. Yampolsky LV, Schauer TMM, Ebert D. Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zoeplankton. Proc Biol Sci. 2014;281:20132744. https://doi.org/10.1098/rspb.2013.2744.

34. Weider LJ, Hebert PDN. Ecological and physiological differentiation among low-artic clones of Daphnia pulex. Ecology. 1987;68:188–98.

35. Agra AR, Soares AMWM, Barata C. Life-history consequences of adaptation to pollution: Daphnia longispina clones historically exposed to copper. Ecotocology. 2011;20:552–62. https://doi.org/10.1007/s10646-011-0621-5.

36. Fisk DL, Latta LC, Knapp RA, Pfrender ME. Rapid evolution in response to introduced predators I: rates and patterns of morphological and life-history trait divergence. BMC Evol Biol. 2007. https://doi.org/10.1186/1471-2148-7-22.

37. Teschner M. Effects of salinity on the life history and fitness of Daphnia magna: variation within and between populations. Hydrobiol. 1995;307:33–41.

38. Miner BE, Kerr B. Adaptation to local ultraviolet radiation conditions among nonturf Daphnia populations. Proc Biol Sci. 2022;278:1306–13. https://doi.org/10.1098/rspb.2020.1663.

39. Santos JL, Ebert D. Trehalose provisioning in Daphnia resting stages reflects local adaptation to the harshness of diapause conditions. Biol Lett. 2012;18:20210615. https://doi.org/10.1098/rbl.2011.0615.

40. Kerfoot WC, Robbins JA, Weider LJ. A new approach to historical reconstruction: Combining descriptive and experimental paleolimnology. Limnol Oceanogr. 1999;44:1322–47. https://doi.org/10.4319/lo.1999.44.4.1322.

41. Orsini L, Schwenk K, De Meester L, Colbourne JK, Pfrender ME, Weider LJ. The evolutionary time machine: using dormant propagules to forecast how populations can adapt to changing environments. Trends Ecol Evol. 2013;28:274–82. https://doi.org/10.1016/j.tree.2013.01.009.

42. Cuyas C, De Meester L, Colbourne JK, Brendonck L, Verschure D, Volkvaert F. Rapid, local adaptation of zooplankton behavior to changes in environmental stressors: the influence of salinity on host-parasite interactions during diapause. Ecol Lett. 2008;11:919–28. https://doi.org/10.1111/j.1461-0248.2008.01263.x.

43. Pulkkinen K. Microparasite transmission to Daphnia magna decreases in the presence of conspecifics. Oecologia. 2007;154:45–53.

44. Lass S, Ebert D. Apparent seasonality of parasite dynamics: analysis of cyclic prevalence patterns. Proc Biol Sci. 2006;273:199–206.

45. Frederiksen M, Ameline C, Bourgeois Y, Voght F, Savola E, Andras J, Engelstadter J, Ebert D. A two-locus system with strong epistasis underlies rapid parasite-mediated evolution of host resistance. Mol Ecol Biol. 2021;38:1512–28. https://doi.org/10.1007/molbev/mnsa.0113.

46. Ihara R, Ben-Ami F. Host age modifies within-host parasite infection, virulence and reproduction. J Anim Ecol. 2015;84:1018–28. https://doi.org/10.1111/1365-2652.12352.

47. Ihara R, Routtu J, Ben-Ami F. Host age modifies within-host parasite infection. Biol Lett. 2015;11:20150131. https://doi.org/10.1098/rsbl.2015.0131.

48. Ben-Ami F, Rigaud T, Ebert D. The expression of virulence during double infections by different parasites with conflicting host exploitation and transmission strategies. J Evol Biol. 2011. https://doi.org/10.1111/j.1420-9101.2011.02264.x.

49. Manzi F, Halle S, Seemann L, Ben-Ami F, Wolinska J. Sequential infection of Daphnia magna by a gut microsporidium followed by a haemolymph yeast decreases transmission of both parasites. Parasitology. 2021;148:1556–77. https://doi.org/10.1017/S0031182021001384.

50. Duffy MA, Hall SR. Selective predation and rapid evolution can jointly dampen effects of virulent parasites on Daphnia Populations. Am Nat. 2008;171:499–510. https://doi.org/10.1086/528998.

51. Hall MD, Vetterg A, Ebert D. Interactions between environmental stressors: the influence of salinity on host-parasite interactions between Daphnia magna and Pasteuria ramosa. Oecologia. 2013;171:789–96. https://doi.org/10.1007/s00442-012-2452-3.

52. Zbinden M, Haag CR, Ebert D. Experimental evolution of field populations of Daphnia magna in response to parasite treatment. J Evol Biol. 2008;21:1088–1078.

53. Alternatt F, Ebert D. Genetic diversity of Daphnia magna populations enhances resistance to parasites. Ecol Lett. 2008;11:918–28. https://doi.org/10.1111/j.1461-0248.2008.01203.X.

54. Pulkkinen K. Microparasite transmission to Daphnia magna decreases in the presence of conspecifics. Oecologia. 2007;154:45–53.

55. Siss L, Ebert D. Apparent seasonality of parasite dynamics: analysis of cyclic prevalence patterns. Proc Biol Sci. 2006;273:199–206.

56. Frederiksen M, Ameline C, Krebs M, Hussy B, Fields PD, Andras JP, Ebert D. Infection phenotypes of a coevolving parasite are highly diverse, structured, and specific. Evolution. 2021;75:2540–54. https://doi.org/10.1111/evol.14323.

57. Auld SKJR, Brand J. Simulated climate change, epidemic size, and host evolution across host-parasite populations. Glob Change Biol. 2017;23:5045–53. https://doi.org/10.1111/gcb.13769.

58. Haag KL, Larsson JRF, Refardt D, Ebert D. Cytological and molecular description of Hamiltosporidium taeniarmeninsis gen. et sp nov, a microsporidian parasite of Daphnia magna, and establishment of Hamiltosporidium magnivora comb. nov. Parasitology. 2011;138:447–62. https://doi.org/10.1017/S0031182011001393.

59. Haag KA, Pombert JF, Sun YK, de Albuquerque NR, Battaillier B, Fields P, Lopes TF, Ebert D. Microsporidia with vertical transmission were likely shaped by nonadaptive processes. Genome Biol Evol. 2020;12:3599–614. https://doi.org/10.1093/gbe/evz270.
66. Kirk D, Luijckx P, Stanic A, Krokosek M. Predicting the thermal and allo-
EvoDevo           (2022) 13:16
67. Lohr JN, Laforsch C, Koerner H, Wolinska J. A
68. Toenshoff ER, Fields PD, Bourgeois YX, Ebert D. The end of a 60-year
69. Andras JP, Fields PD, Du Pasquier L, Fredericksen M, Ebert D. Genome-
70. Lange B, Kaufmann AP, Ebert D. Genetic, ecological and geographic
72. Bento G, Fields PD, Duneau D, Ebert D. An alternative route of bacterial
75. Herzog Q, Rabus M, Ribeiro BW, Laforsch C. Inducible defenses with a
76. Gerber N, Kokko H, Ebert D, Booksmythe I.
79. Weiss LC, Albada B, Becker SM, Meckelmann SW, Klein J, Meyer M,
82. Horstmann M, Tollrian R, Weiss LC. Thwarting predators? A three-
83. Ebert D. The trade-off between offspring size and number in Daphnia magna: the influence of genetic, environmental and maternal effects. Arch Hydrobiol. 1993;90:453–73.
84. Moenickes S, Richter O, Picov R. Approaching the evolutionary advan-
to the freshwater crustacean Daphnia. PLoS ONE. 2021. https://doi.org/10.1371/journal. pone.0254263.
85. Ebert D. The trade-off between offspring size and number in Daphnia magna: the influence of genetic, environmental and maternal effects. Arch Hydrobiol. 1993;90:453–73.
86. OECD. Test No. 022: Daphnia sp. Acute Immobilisation Test, 2004.
87. OECD. Test No. 211: Daphnia magna Reproduction Test, 2012.
107. Kumagai H, Nakanishi T, Matsuura T, Kato Y, Watanabe H. CRISPR/Cas-mediated knock-in via non-homologous end-joining in the crustacean Daphnia magna. PLoS ONE. 2017. https://doi.org/10.1371/journal.pone.0186112.

108. Naitou A, Kato Y, Nakanishi T, Matsuura T, Watanabe H. Heterodimeric TALENs induce targeted heritable mutations in the crustacean Daphnia magna. Biol Open. 2015;4:364–9. https://doi.org/10.1242/bo.2014.9738.

109. Nakanishi T, Kato Y, Matsuura T, Watanabe H. TALEN-mediated knock-in via non-homologous end-joining in the crustacean Daphnia magna. Sci Rep. 2016. https://doi.org/10.1038/srep36252.

110. Arao T, Kato Y, Nong QD, Yamamoto H, Watanabe H, Matsuura T, Tatarazako N, Tani K, Okamoto A, Matsumoto T, et al. Production of genome-edited Daphnia for heavy metal detection by fluorescence. Sci Rep. 2020. https://doi.org/10.1038/s41598-020-78572-z.

111. Schumpert CA, Dudycha JL, Patel RC. Development of an efficient RNA interference method by feeding for the microcrustacean Daphnia. BMC Biotechnol. 2015. https://doi.org/10.1186/s12896-015-0209-x.

112. Adhitama N, Matsuura T, Kato Y, Watanabe H. Monitoring ecdysteroid metabolism using genetically encoded reporter gene in Daphnia magna. Mar Environ Res. 2018;140:375–81. https://doi.org/10.1016/j.marenvres.2018.07.003.

113. Kato Y, Nakanishi T, Watanabe H. Genome-editing in the Crustacean Daphnia magna using CRISPR/Cas and TALEN systems. In: Appasani K, Church GM, editors. Genome editing and engineering: from TALEns, Zfns and Crisprs to molecular surgery. Cambridge: Cambridge University Press; 2018.

114. Rivetti C, Campos B, Pina B, Raldúa D, Kato Y, Watanabe H, Barata C. Tryptophan hydroxylase (TPH) loss of function mutations induce growth and behavioral defects in Daphnia magna. Sci Rep. 2018. https://doi.org/10.1038/s41598-018-19778-0.

115. Nguyen ND, Matsuura T, Kato Y, Watanabe H. DNMT3.1 controls trade-offs between growth, reproduction, and life span under starved conditions in Daphnia magna. Sci Rep. 2021. https://doi.org/10.1038/s41598-021-86578-4.

116. Routtu J, Ebert D. Genetic architecture of resistance in Daphnia hosts against two species of host-specific parasites. Heredity. 2015;114:241–8. https://doi.org/10.1038/hdy.2014.97.

117. Czyzponka T, Fields PD, Routtu J, van den Berg E, Ebert D, De Meester L. The genetic architecture underlying diapause termination in a planktonic crustacean. Mol Ecol. 2019;28:998–1008. https://doi.org/10.1111/mec.15003.

118. Ravindran SP, Luneburg J, Gottschlich L, Tams V, Cordellier M. Daphnia stressor database: taking advantage of a decade of Daphnia -omics’ data for gene annotation. Sci Rep. 2019. https://doi.org/10.1038/s41598-019-47226-0.

119. Schwarzenberger A, Chen LX, Weiss LC. The expression of circadian clock genes in Daphnia magna diapause. Sci Rep. 2012. https://doi.org/10.1038/srep0077650.

120. Brun NR, Fields PD, Hornsfield S, Mirbahai L, Ebert D, Colbourne JK, Fent K. Mixtures of aluminum and indium induce more than additive phenotypic and toxicoecogenomic responses in Daphnia magna. Environ Sci Technol. 2019;53:1639–49. https://doi.org/10.1021/acs.est.8b05457.

121. Molinier C, Reisser CM, Fields PD, Segard A, Galimov Y, Haag CR. Evolution of gene expression during a transition from environmental to genetic sex determination. Mol Biol Evol. 2019;36:1551–64. https://doi.org/10.1093/molbev/msz123.

122. Wilde MV, Brehm J, Schwarzer M, Stockl JB, Laforsch C, Frohlich T. Improving the proteome coverage of Daphnia magna—implications for future ecotoxicoproteomics studies. Proteomics. 2022;22:e2100289. https://doi.org/10.1002/pmc.202100289.

123. Zaffagnini F, Sabelis M. Karyologic observations on the maturation of the summer and winter eggs of Daphnia pulex and Daphnia middendorffiana. Chromosoma. 1972;69:193–203.

124. Zaffagnini F. Reproduction in Daphnia Memsit Itl Idriobl (Memori Dell’Istituto Italiano di Idriobiologia DottMaco De Marchi). 1987;64:245–284.

125. Tsuchiya D, Eads BD, Zolan ME. Methods for meiotic chromosome preparation, immunofluorescence, and fluorescence in situ hybridization in Daphnia pulex. In: Keeney S, editor. Meiosis, vol. 2. Heidelberg: Springer; 2009. p. 235–49.

126. Cristescu MEA, Colbourne JK, Radivoj J, Lynch M. A micro satellite-based genetic linkage map of the waterflea, Daphnia pulex: on the prospect of crustacean genomics. Genomics. 2006;88:415–30.

127. Girbet G, Edgecombe GD. Current Understanding of Ecdysozoa and its internal phylogenetic relationships. Integr Comp Biol. 2017;57:455–66. https://doi.org/10.1093/icb/icx072.

128. Martin-Durán JM, Vellutini BC. Introduction: young approaches to animal evolution. In: Martin-Durán JM, Vellutini BC, editors. Old questions and young approaches to animal evolution. Cham: Springer; 2019. p. 1–13.

129. Andrew DR. A new view of insectecrustacean relationships II. Inferences from expressed sequence tags and comparisons with neural cladistics. Arthropod Struct Dev. 2011;40:289–302. https://doi.org/10.1016/j.asd.2011.02.001.

130. von Reumont BM, Jenner RA, Wills MA, Dell’Ampio E, Pass G, Ebersberger I, Meyer B, Koenemann S, Iliffe TM, Stamatakis A, et al. Pancrustacean phylogeny in the light of new phylogenomic data support for remipedia as the possible sister group of hexapoda. Mol Biol Evol. 2012;29:1031–45. https://doi.org/10.1093/molbev/mss270.

131. Duneau D, Luijckx P, Ben-Ami P, Laforsch C, Ebert D. Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host-parasite interactions. BMC Biol. 2011:9:1. https://doi.org/10.1186/1741-7007-9-11.

132. Laforsch C, Tollrian R. Extreme helmet formation in Daphnia cucullata induced by small-scale turbulence. J Plank Res. 2004;26:81–7.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

At BMC, research is always in progress.

Learn more: biomedcentral.com/submissions