# High genetic variation in resting-stage production in a metapopulation: Is there evidence for local adaptation?

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**Local adaptation is a key process for the maintenance of genetic diversity and population diversification. A better understanding of the mechanisms that allow (or prevent) local adaptation constitutes a key in apprehending how and at what spatial scale it occurs. The production of resting stages is found in many taxa and reflects an adaptation to outlast adverse environmental conditions. *Daphnia magna* (Crustacea) can alternate between asexual and sexual reproduction, the latter being linked to dormancy, as resting stages can only be produced sexually. In this species, on a continental scale, resting-stage production is locally adapted—that is, it is induced when the photoperiod indicates the imminence of habitat deterioration. Here, we aimed to explore whether selection is strong enough to maintain local adaptation at a scale of a few kilometers. We assessed life-history traits of 64 *D. magna* clones originating from 11 populations of a metapopulation with permanent and intermittent pool habitats. We found large within- and between-population variation for all dormancy-related traits, but no evidence for the hypothesized higher resting-stage production in animals from intermittent habitats. We discuss how gene flow, founder events, or other forms of selection might interfere with the process of local adaptation.**

**KEY WORDS: *Daphnia magna*, local adaptation, male production, metapopulation, resting-stage, sex-allocation.**

Many organisms are found in habitats that are seasonally variable and for part of the year unsuitable for physiological active life stages to survive. Most of these organisms, from microorganisms (Lennon and Jones 2011) to perennial plants (Rohde and Bhalerao 2007; Linkies et al. 2010) and animals (Carlisle and Pitman 1961; Moran 1992; Schöder 2005; Evans and Dennehy 2005), outlive harsh conditions by entering dormancy. Across taxa, dormancy can occur at any developmental stage, and organisms use different environmental cues, such as changes in temperature, photoperiod,

or food resources, to predict the deterioration of their habitat and enter dormancy (Hairston and Olds 1984; Moran 1992; Alekseev and Lampert 2001; Simon et al. 2002; Gilbert and Schroder 2004; Koch et al. 2009).

Dormancy, however, is not without cost. A large number of species show phenotypic plasticity regarding the completion of their life cycle, and experience a fundamental trade-off between the production of directly developing stages and resting stages. An example is the trade-off between asexual reproduction and

resting-stage production through sexual reproduction that occurs for plants (vegetative reproduction vs. seed production, Rohde and Bhalerao 2007) and facultative parthenogenetic taxa such as algae, aphids, rotifers, and cladocerans (John 1994; Gilbert and Schreiber 1998; Simon et al. 2002; Cáceres and Tessier 2004). In such species, the production of resting stages by sexual reproduction is costly not only because it interferes with asexual reproduction, but also because it often requires the production of males beforehand to ensure fertilization. Thus, when environmental conditions are favorable, individuals that invest in resting stages are disadvantaged compared with those that reproduce asexually (Lynch 1983). Conversely, if a habitat deteriorates, a failure in switching to resting-stage production would result in high costs and immediate fitness loss for the individual (Deng 1996). Due to this trade-off, the induction of dormancy is predicted to be under strong selection (Levins 1968). Yet the costs and benefits of dormancy will depend on environmental factors, such as seasonality and the frequency and extent of the harsh conditions. Our expectation, therefore, is that selection for dormancy induction should vary in space and be subject to local adaptation (Hairston and Olds 1984).

Typically, local adaptation is expected when populations of a given species are found in ecologically contrasting habitats and experience locally different selection that exceeds the homogenizing force of gene flow and random effects of genetic drift (Kawecki and Ebert 2004). As local adaptation is an important way to produce and maintain genetic diversity across populations (Mitchell-Olds et al. 2007), a wealth of theoretical (Kawecki and Ebert 2004; Bierne et al. 2011; Blanquart et al. 2013; Tiffin and Ross-Ibarra 2014) and empirical studies have identified the mechanisms that shape local adaption and link phenotypic diversity to local selection. Examples of local adaptation have indeed been reported in a large number of species and for various traits (Michalak et al. 2001; Capelle and Neema 2005; Eränen 2006; Korol et al. 2006; Manceau et al. 2011; Kesari et al. 2012; Kreyling et al. 2014; Muir et al. 2014; Yampolsky et al. 2014). Most of these studies focused on populations separated by distances exceeding the typical dispersal capacity of the organisms (Richardson et al. 2014). At large geographic scales, the chances of detecting local adaptation are relatively high, as gene flow is expected to be low (see Ebert 1994 for a gradient of geographic distances). Thus, even though such cases represent compelling examples of population diversification, they provide little information on the interplay among local selection, gene flow, and phenotypic variation. To this end, studies focusing on microgeographical adaptation, which is adaptation within the dispersal range of the organism, can be more informative as they allow one to examine the strength of selection in the context of fine-scale diversification (Capelle and Neema 2005; Laine 2005; Kavanagh et al. 2010; Manceau et al. 2011; Fitzpatrick et al. 2014; García-Navas et al. 2014; Moody et al. 2014; Langin et al. 2015).

Freshwater habitats represent ideal systems to tackle the question of microgeographical adaptation as the characteristics of local ponds can vary drastically within the same geographic area. This is especially true regarding water permanency: some ponds may completely desiccate in summer, whereas other ponds may provide a permanent habitat throughout the growth period. Several studies showed that the likelihood of pond desiccation strongly impacts the incidence of dormancy of populations of aquatic invertebrates (Cáceres and Tessier 2004; Gilbert and Schroder 2004; Guidetti et al. 2011). In our model organism, the facultative parthenogenetic crustacean *Daphnia magna*, we previously demonstrated that populations are locally adapted with respect to the timing and extent of dormancy (Roulin et al. 2013). The production of resting stages (hereafter called "ephippia") correlated most strongly with the photoperiod that best indicated the imminence of water disappearance, for example, summer draught or winter freezing. Populations from rock pool in Northern Europe, however, produced high levels of resting stages at any photoperiod, a pattern predicted from the combined factors of a high likelihood of summer draught and winter freezing (Roulin et al. 2013). These populations are the subjects of the current study.

In this follow-up study, our aim was to assess whether selection is strong enough to produce and maintain local adaptation of dormancy induction at a geographic scale of a few kilometers, a scale on which migration, and thus gene flow, has been observed (Pajunen 1986; Pajunen and Pajunen 2003; Haag et al. 2006; Ebert et al. 2013). To do so, we used a well-studied Finnish metapopulation (Pajunen 1986; Pajunen and Pajunen 2003; Haag et al. 2006; Ebert et al. 2013) that consists of subpopulations inhabiting a multitude of freshwater rock pools on islands in the Baltic Sea (Pajunen and Pajunen 2003; Altermatt et al. 2009; Altermatt and Ebert 2010). All pools freeze in winter, but in summer, smaller pools undergo recurrent desiccation whereas deeper pools have permanent water (Altermatt and Ebert 2008), producing a patchwork of locally divergent selection for dormancy induction. Thus, populations inhabiting summer-dry (intermittent) pools are expected to produce resting stages and males already early in the summer season (marked by increasing photoperiod), before pool desiccation. On the other hand, populations from permanent pools are expected to produce few males and resting stages in early summer because those are not required to survive the season.

Here, we used 64 clones of *D. magna* originating from five intermittent and six permanent rock pool populations of the metapopulation and tested this hypothesis under controlled environmental conditions. We used increasing photoperiod as a signal for early summer condition and predicted that *Daphnia* from intermittent habitats will produce more resting stages under these

conditions than those from permanent pools. We detected high within- and between-population variations for the production of ephippia and males, but no evidence for local adaptation to habitat type. We discuss the role of gene flow and founder events in shaping local gene pools and suggest that selection for balanced sex allocation might also influence local adaptation.

## Methods

### INITIAL CULTURE

We chose populations from 11 rock pools (pool number codes: FS-31, N-86, SK-1, FUN-13, LA-10, LA-18, SKW-1, SKW-2, LON-1, SK-45, HA-1) located on nine different islands near Tvärminne Zoological Station, South-Western Finland (59°8'N, 23°2'E). These islands have been subject to extensive field study since 1982, wherein data regarding pool ecology and *Daphnia* sp. population presence/absence have been collected twice per year (Pajunen 1986; Pajunen and Pajunen 2003; Ebert et al. 2013).

We collected *D. magna* ephippia of each population in July 2012 and hatched them in the laboratory following the protocol of Sheikh-Jabbari et al. (2014). The 11 populations were chosen based on the following criteria: Only populations that have existed for at least eight years (so that selection has had time to act) and only populations from rock pools on either extreme of the water permanency scale (according to the pool desiccation model by Altermatt et al. 2009) were included. "Permanent" pools are rock pools that never or only very rarely completely desiccate during summer (pools FS-31, SKW-2, N-86, SK-1, FUN-13, and LA-10) and "intermittent pools" are rock pools that frequently desiccate completely in summer (pools SKW-1, LON-1, LA-18, SK-45, and HA-1). All pools freeze in winter. Finally, pools were chosen to be approximately evenly distributed across the study area, to avoid a spatial autocorrelation with regard to pool type (permanent vs. intermittent, Fig. 1)—for example, by avoiding two pools of the same type on the same island. To avoid including full- or half-sib clones, only one hatchling per ephippium case was kept. From each population, six hatchlings were randomly selected and isolated to establish an isofemale line (a "clone" consisting of the hatchling and its clonal descendants).

In these chosen rock-pools, all but one of the studied populations (LA-18) were infected with the microsporidian parasite *Hamiltosporidium tvaerminnensis* (formerly called *Octosporea bayeri*, Haag et al. 2011), which is transmitted through the eggs in the ephippia. This parasite is known to bias offspring production toward male production in *D. magna* (Roth et al. 2008). Therefore, we used fumagilin B to cure all clones by blocking vertical disease transmission (Zbinden et al. 2005). In brief, we raised for each clone (including LA18 clones) a large number of immature females in artificial *Daphnia* medium (AdaM, Klüttgen et al. 1994) containing fumagilin B. Immediately after the females
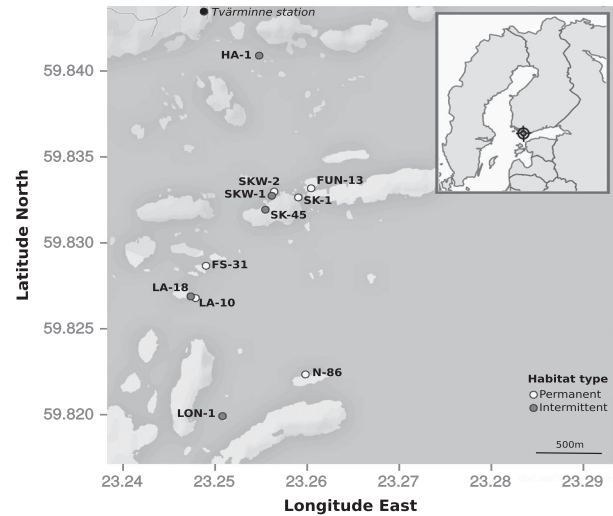


**Figure 1.** Geographic location of rock pool populations sampled in this study on the islands of Tvärminne archipelago.

started to reproduce asexually, we transferred their offspring into fresh medium and raised them for one month before checking for the presence of the parasite. The cured clones were cultured in the laboratory for several generations and kept at 20°C with 16 h of light per day. Two clones were lost after the treatment, resulting in 64 clones in total.

All animals were kept under standardized culture conditions in the laboratory, unless mentioned otherwise: 20°C, 16:8 h light:dark cycle, ADaM (Klüttgen et al. 1994), and chemostat grown green algae (*Scenedesmus obliquus*) as the sole food. For the experiment, animals were fed with four million algae cells per day and kept in 100-mL jar.

### EXPERIMENTAL DESIGN

For each of the 64 clones, we started 12 independent replicates using one- to three-day-old females raised individually in 100-mL jars filled with 80-mL medium. To minimize variation in maternal effects, females of the third generation were used (i.e., replicates were grown for two preexperimental generations under identical conditions as during the experiment). We then distributed the replicates haphazardly across trays and shelves in an incubator to minimize bias due to position effects. Throughout the experiment, we moved trays among and within shelves every third day to further reduce position effects. After two weeks (starting from maturity of most females), we changed the photoperiod in the incubator following the design of Roulin et al. (2013). To mimic the spring-to-summer change in photoperiod, day length was gradually increased every day over six weeks from 16 to 22 h of light per day. Replicates were split into two sets of six.

The first set of six replicates was used to assess asexual offspring production. For this, initial females were transferred

individually into fresh medium once a week. Offspring produced were sexed and counted every week. We obtained male proportion and total offspring production data at six time points for each replicate. In addition, after week 6, we continued transferring the mothers until they died to assess the lifespan of each female. During this period, offspring were not recorded as a decreasing number of replicates were still alive and even a lower number still produced offspring.

The second set of six replicates was used to assess ephippia production only. Based on previous experiments (Roulin et al. 2013), we knew that ephippia are produced in crowded conditions. Thus, initial females were allowed to reproduce and build up clonal populations in the jars. No offspring were removed so that the maximum carrying capacity could be reached within the jars. Ephippia production was assessed every week. As ephippia production is delayed compared with asexual male production, we monitored these replicates for a total of eight weeks.

### DATA ANALYSIS

For each replicate, the cumulative number of ephippia, proportion of males (i.e., sons/total offspring production), and total asexual offspring (sons + daughters) were calculated. As the number of total offspring produced until week 6 was positively correlated with lifespan at the clone level (see Results), we standardized total offspring productions by lifespan. Male proportion was not corrected for lifespan because no correlation was observed between male proportion and lifespan at the clone within population level (see Results). To approximate normal distribution of residuals, a square-root transformation was applied to ephippia production. No transformation was applied to the other traits because no obvious deviation from normal distribution was observed.

Even though populations were chosen to be evenly distributed with regard to pool type, we tested whether ephippia and male production were correlated with geographic distances. Using mantel tests, we found that there was no correlation between geographic distances between pools and differences in ephippia production or male proportion in our data (data not shown). Therefore, we did not include data related to potential local network structures in our models. Differences in ephippia and total offspring production, as well as in male proportion and lifespan, between pool types (permanent vs. intermittent) were tested using linear mixed-effect models. Note that male proportion data at the clone level were mostly between 0.2 and 0.5 and were not approaching the 0–1 limits. To ease comparisons between models with different response variables, we therefore decided to use a linear mixed model rather than a logistic mixed model for male proportion as well. We nevertheless tested and confirmed that with a mixed-effect logistic regression, the habitat type has no effect on male proportion (data not shown). For each trait, we built a full linear mixed-effect model in which the pool type (permanent vs. intermittent) was entered as a fixed factor and population and clone were used as random factors, with clone being nested within population, and population being nested within habitat type. The choice of using both population and clone as random factors was motivated by comparing different random factors (population only, clone only, both) with the Akaike Information Criterion (AIC) and picking the best combination of random factors. We then assessed the significance of pool type using a likelihood ratio test that compared the models with and without pool type. Standard deviations of the population and clone effects were extracted and used to calculate variance components as described in Crawley (2007).

We were also interested in the association among life-history traits. Specifically, for correction purposes in the statistical analysis (see above), we wanted to test whether females that live longer produce significantly more male (in proportion) and offspring. In addition, to test for a potential association between ephippia and male production, we wanted to assess whether clones that produce ephippia at a high rate produced males in a lower proportion.

With our design, the association between two traits can be observed both at the population and clone (within populations) levels, and classical mixed-models do not allow them to be distinguished. We therefore used the within-population centering method of van de Pol and Wright (2009) to distinguish and test for the significance of the within- ($\beta W$) and between-population ($\beta B$) effects on the association. Eventually, if significant, we were also interested in testing whether the tendency of the association at the between- and within- population levels was the same—that is, whether the within-population slopes paralleled to the between-population slope. To do so, we tested whether the difference between the between-population and the within-population effects ($\beta B - \beta W$) deviated from zero. If $\beta B - \beta W$ does not significantly deviate from zero, then within- ($\beta W$) and between-population ($\beta B$) effects are considered to be the same.

All models were run using a linear mixed-effect model in which (depending on the association tested) total offspring production and male proportion were entered as dependent variables whereas lifespan or ephippia production were entered as predictor variables. Population and clone were entered as random effects. Note that, as in the previous analysis, the pool-type effect on the between- and within-population interactions was tested but not integrated in the final analysis because it was not significant.

All statistical analyses were performed with R, version 3.1.0 (R Development Core Team 2013). Figure 1 was realized using maps package (Becker and Wilks 2014). Mixed-effect model analyses were performed with nlme package (Pinheiro et al. 2014). Mantel tests were performed with ade4 package (Dray and Dufour 2007).

**Table 1.** Statistics of pool-type, population, and clone effects for the four life-history traits studied.

| | Fixed factor effects | Random factor effects | | | | Residual effects |
| | Pool type | Population | | Clone | | |
| Trait | *P*-value | *P*-value | Variance component | *P*-value | Variance component | Variance component |
|---|---|---|---|---|---|---|
| Ephippia prod. | 0.66 | <0.0001 | 25.6 | <0.0001 | 33 | 41.4 |
| Total offspring prod. | 0.75 | <0.0001 | <1 | <0.0001 | 22.9 | 77.1 |
| Male proportion | 0.18 | <0.0001 | 8.4 | 0.01 | 9.3 | 82.2 |
| Lifespan | 0.68 | <0.0001 | 14.3 | 0.004 | 10.5 | 75.2 |

*P*-values were calculated using mixed-effect model Akaike Information Criterion. Variance components of the population and clone effect are expressed as percentages.
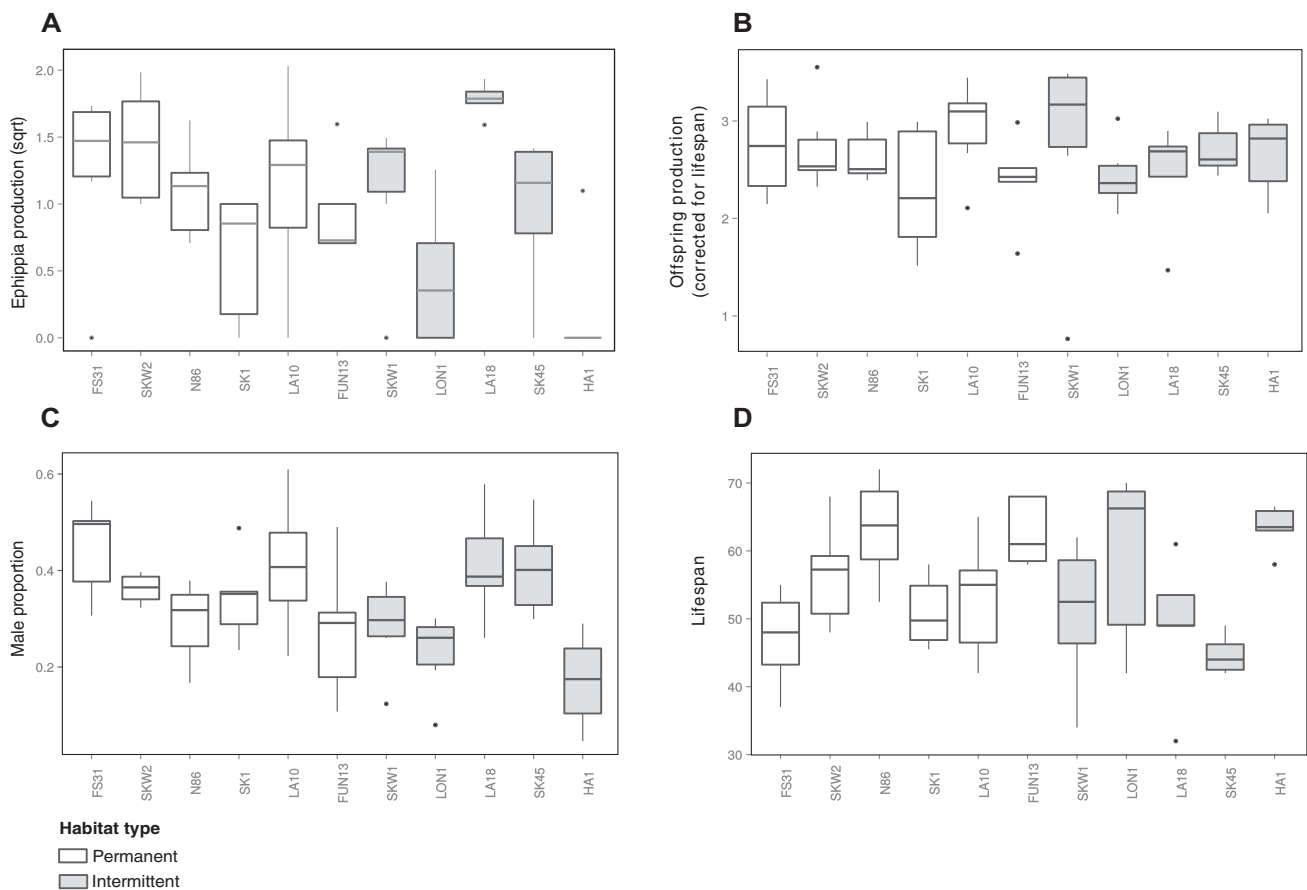


**Figure 2.** Boxplots for each population for the four life-history traits measured. **(A)** Ephippia production. **(B)** Total offspring production. **(C)** Male proportion. **(D)** Lifespan. Ephippia production is square-root transformed. Total offspring production is corrected for lifespan. Boxplots show the median, upper, and lower quartiles. Black dots represent outliers.

# Results

## ANALYSIS OF HABITAT, POPULATION, AND CLONE EFFECTS ON SEX ALLOCATION

To test for local adaptation in the four traits studied, we estimated the differences in median trait values between pool types (permanent vs. intermittent), as well as the amount of variation explained by populations and *Daphnia* clones. The results of the mixed models showed that the pool type had no effect on any of the traits measured (Table 1). Thus, there is no evidence for local adaptation with regard to pool permanency. In contrast, we found a significant population effect for ephippia production (25.6% of the variance explained by the population effect), male proportion

(8.4%), and lifespan (14.3%). The population effect was also significant for total offspring production, but it explained a smaller part of the variance (Table 1, Fig. 2).

We also observed significant clone effects within populations for all traits, explaining between 9 and 33% of the variance (Table 1). Of the four traits studied, ephippia production was the one for which the largest part of the variance could be explained by the sum of clone and population effects (~60%), whereas male proportion was the one for which the smallest part of the variance was explained (~18%).

### ASSOCIATION AMONG LIFE-HISTORY TRAITS

To investigate associations among traits underlying dormancy, we compared and contrasted the within- ($\beta W$) and between-population ($\beta B$) components of the association. At the population level, we observed a positive correlation between lifespan and total offspring production. This association was also significant at the clone within-population level. There was no significant difference between the within- and between-population trends as the within-population slopes were parallel to the between-populations slopes ($\beta B - \beta W$) not significant from zero (Table 2, Fig. 3A). Thus, females from clones that lived longer produced more offspring in total than females from clones with a shorter lifespan. We corrected the total offspring production of each female by its lifespan in the mixed-effect model analysis (see Methods). On the other hand, we observed a negative association between male proportion and lifespan, but only at the between-population level (Table 2, Fig. 3B). At the clone level, the proportion of male produced was not dependent of the lifespan of the female. Therefore, we did not correct male proportion by lifespan. We did not test for the consistency of the slopes at the between- and within- population levels, because the within-population effect $\beta W$ was not significant.

Similarly, the association between ephippia production and male proportion was significant only at the between-population level (Table 2, Fig. 3C), indicating that overall, populations that produced more ephippia also showed a higher male proportion. Within populations, these traits were not correlated across clones. Here again, because the within-population effect $\beta W$ was not significant, we did not test for the consistency of the slopes at the between- and within-population levels.

## *Discussion*

Dormancy induction is expected to be under strong selection and therefore to reveal a pattern of local adaptation (Levins 1968). This is also the case of *Daphnia* living in seasonal environments (Cáceres and Tessier 2004; Roulin et al. 2013). Understanding at what scales phenotypic divergence and local adaptation occur constitutes a key step in our comprehension of the evolution of

life-history traits associated with dormancy. If populations are sufficiently isolated (little or no gene flow) and are large and old enough, local adaptation might occur at the scale of the local deme. However, adaptation might take place at a larger scale, such as the metapopulation level, if demes are strongly connected by gene flow and local populations have a high turnover rate (Hanski et al. 2011). In our previous study, we showed that, at a European scale, dormancy induction in *D. magna* is adapted to the local habitat, a pattern we did not recover here at a much finer scale.

Despite substantial phenotypic variation among and within populations (Fig. 2), the habitat type (permanent vs. intermittent) had neither an effect on traits associated with sexual reproduction nor on traits associated with asexual reproduction. The strong genetic variance components on the population and clone levels observed for the four traits studied confirmed that we sampled different genotypes per population and that our experimental design and our sampling effort were sufficient to detect genetic variation. As a similar design (with fewer genotypes per population) allowed us to detect local adaptation on a larger geographic scale in a previous study (Roulin et al. 2013), we conclude that local adaptation of sex induction does not occur at the scale of a few kilometers, or at least not with regard to habitat permanency. These finding are in agreement with Hanski et al. (2011), who postulated that in populations with fast turnover, adaptation might occur at the scale of the metapopulation, as species might adapt to a larger network rather than to local patches. Different mechanisms linked to the dynamics of the metapopulation itself could explain why local adaptation takes place at a larger scale than what we expected. We discuss those in the light of strength of selection, gene flow, and genetic drift.

### STRENGTH OF SELECTION

In this metapopulation, local extinction rates are reported to be high, approaching on average 16% per year (Pajunen and Pajunen 2003; Altermatt and Ebert 2010) and resulting in a low average population life expectancy (Pajunen and Pajunen 2003, Altermatt et al. 2009). Even though the recorded population lifespans for the two pool groups did not differ from each other in our experiment, small intermittent pools have been shown to harbor shorter-lasting populations under natural conditions (Pajunen and Pajunen 2003; Altermatt et al. 2009). If selection is not strong enough, local adaptation could thus be restrained by the limited amount of time available for the population to adapt to a given pool environment. This fast turnover, however, does not seem to be a limitation, as an experimental evolution study conducted within this natural rock pool system showed that populations can adapt rapidly (within two years) to specific environmental conditions (Zbinden et al. 2008). In addition, and as shown in different species, (Cáceres and Tessier 2004; Smith and Snell 2012; Roulin et al. 2013), selection on sex allocation and dormancy induction is expected to

**Table 2.** Statistics for population (β*B*) and clone within-population (β*W*) effects on the associations among life-history traits.

| | *P*-values | | |
| --- | --- | --- | --- |
| Association tested | Population effect (β*B*) | Clone within-population effect (β*W*) | β*B* − β*W* |
| Total offspring production (raw)~lifespan | 0.002 | <.0001 | 0.3 |
| Male proportion~lifespan | 0.001 | 0.82 | NI |
| Male proportion~ephippia production | 0.03 | 0.5 | NI |

The consistency of the association at the population and clone levels (β*B* – β*W*) was not estimated if β*W* was not significant.
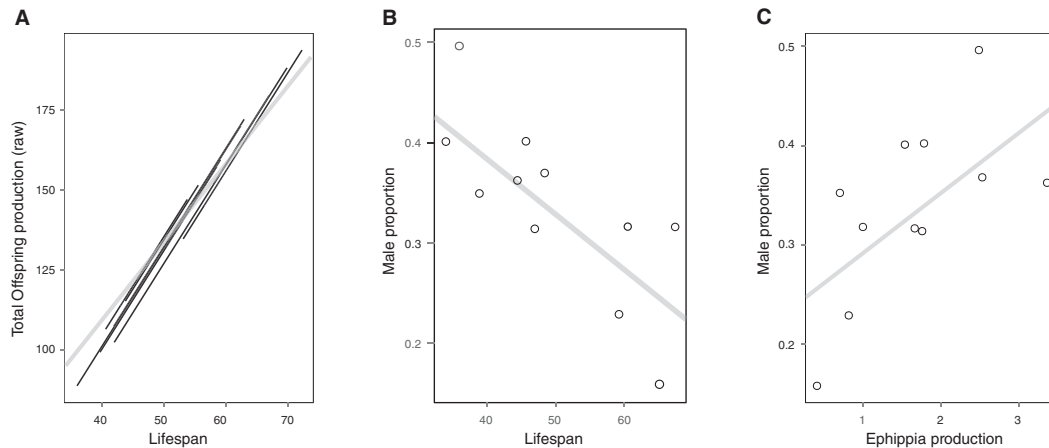


**Figure 3.** Scatter plots and regression lines showing associations among life-history traits. (A) Total offspring production versus lifespan. (B) Male proportion versus lifespan. (C) Male proportion versus ephippia production. The thick gray line shows the regression line on the population level. Thinner black lines show regression line for each population when the association between the two traits was significant at the within-population level. When the association was not significant at the within-population level, only points representing the median value per population were plotted.

be strong in such ephemeral habitats (Smith and Snell 2012). As we carefully chose pools for which the presence of *D. magna* had been recorded for at least eight years (mostly longer), selection should have had time to act. We thus believe that the relatively short lifespan of the populations is unlikely to interfere with local adaptation.

### GENE FLOW AND SOURCE–SINK STRUCTURE

Gene flow may counteract the effect of local selection. Extinctions in our *Daphnia* metapopulation are approximately balanced by colonization (Pajunen and Pajunen 2003). This suggests a high migration rate among populations (Ebert et al. 2013). In *D. magna,* dispersal of ephippia is passive (Maguire 1963) and happens mostly when pools are desiccated (Vanschoenwinkel et al. 2008). Small intermittent pools that are numerically predominant in this metapopulation are believed to produce more emigrants than populations from larger and permanent pools. As a consequence, migration rates between intermittent and permanent pools are likely to be asymmetric, causing the metapopulation to have a source–sink structure (Altermatt and Ebert 2010).

Local adaptation might thus occur with regard to the environmental conditions encountered only in the source habitats, with adaptation to the sink conditions being prevented by recurrent gene flow (Dias and Bataillon 1996; Kennington et al. 2003; Kawecki and Ebert 2004). Such a structure of the metapopulation could explain the absence of local adaptation as suggested in other systems (Kniskern et al. 2011). In this regard, the results from our previous experiment nicely support this hypothesis because Fennoscandian populations (all occurring in rock pools) produced ephippia and males at a high rate compared with the more continental populations tested, in both decreasing and increasing photoperiods (Roulin et al. 2013). In addition, this structure could explain the pattern of phenotypic variation observed. In the metapopulation, gene flow between islands is believed to be limited (Haag et al. 2006). Sinks are therefore likely to receive migrants from surrounding sources located on the same island (Haag et al. 2006). The first process (isolation by distance) could be responsible for the among-population variations detected, whereas the latter (within-island migration from multiple sources) might explain the large within-population variation we observed. With this in mind, we believe that the source–sink structure is an

important factor shaping the evolution of the subpopulations. Other mechanisms could reinforce this process and further prevent local adaptation from taking place.

## INBREEDING, LINKAGE DISEQUILIBRIUM, AND LINKED SELECTION

Another consequence of the extinction/recolonization dynamics discussed above is strong founder events, with an estimated average of 1.7 founder-genotypes per population (Haag et al. 2005). In populations with such frequent population bottlenecks, strong linkage disequilibrium (LD) can build up. This LD is especially pronounced when founder effects are followed by clonal expansion and selection on clones whose entire genome can be seen as a single linkage group (Vanoverbeke and De Meester 1997, de Meeûs and Balloux 2004; Halkett et al. 2005). Both processes may contribute to our results here.

Founder effects and the resulting low local genetic diversity can produce inbreeding and increase genetic differentiation across subpopulations in *D. magna* (Haag et al. 2005). This is known to limit the capacity of the populations to adapt to their new habitat (Boileau et al. 1992; Davis 2009). In addition, inbreeding has a strong impact on fitness in *D. magna,* as it decreases fertility (Haag et al. 2002) and increases mortality rate (Innes and Dunbrack 1993; De Meester and Vanoverbeke 1999). As a consequence, immigrant genotypes have an advantage when they crossbreed with the inbred resident genotypes and produce outbred offspring. This form of hybrid vigor has been demonstrated in this rock pool metapopulation and was shown to elevate the effective migration rate (gene flow) substantially. In extreme cases, the immigrant clones contribute up to 50% of the gene pool in the invaded population (Ebert et al. 2002). This hybrid vigor might result in the reinforcement of the initial LD produced by bottlenecks and result in strong genetic hitchhiking during the phase of clonal competition. This process may interfere with other adaptive processes and thus may slow down adaptation of sex induction.

Besides selection favoring hybrids, we believe that selection for an optimal sex ratio could also slow down local adaptation to environmental conditions. Resting stages can only be produced when males fertilize the ephippial eggs. The two components of sexual reproduction must thus be synchronized, as shown for instance in a Russian population in which ephippia and male production peak simultaneously in September (Galimov et al. 2011). On the other hand, to prevent females from mating with their sons and therefore to limit inbreeding, these functions are expected to be decoupled at the clone level (Innes and Dunbrack 1993; De Meester and Vanoverbeke 1999). This genetic decoupling was also suggested by a quantitative trait locus (QTL) study of male and ephippia production (Roulin et al 2013), in which QTLs for both traits mapped to different regions in the genome and did not show interaction. Thus, selection should optimize a reproductive strategy that maximizes egg fertilization at the population level but limits selfing at the clone level. Due to strong founder events in this metapopulation (Haag et al. 2005, 2006) and the strong variation observed here among clones, populations may at times be far away from balanced male and ephippia production. In such cases, clones investing in the minority function (ephippia or males) would be favored (Hamilton 1967), disregarding other phenotypic features. In support of this hypothesis, we found of a positive association between ephippia and male proportion at the population level, but not at the clone level (Fig. 3C), and strong genetic variation for both traits within populations (Table 1). If selection for optimal sex ratio is stronger than selection for the right timing for dormancy induction, then clonal selection and the resulting LD might further prevent the evolution for adaptation of dormancy induction to pool permanency.

## CONCLUSION

Identifying the conditions that promote local adaptation constitutes a fundamental step in our understanding of population divergence and maintenance of genetic diversity. Although many studies have focused on local adaptation at large geographical scales, more and more studies are revealing the importance of microgeographical adaptation for ecological and evolutionary processes (Skelly 2004; Capelle and Neema 2005; Kavanagh et al. 2010; Manceau et al. 2011; Fitzpatrick et al. 2014). Thus, the question is no longer whether microgeographical adaptation can happen, but what factors favor or prevent it. The study presented here, together with our former work (Roulin et al. 2013), constitutes one of the few that allow the comparison between microgeographical and large-scale adaptation in a given species. Here, we show that microgeographical adaptation is prevented despite large phenotypic variation and putatively high selective pressure. In the current study, the factors that appear to play a role in preventing local adaptation (genetic drift, founder events, inbreeding, source–sink structure) all have a direct link to metapopulation processes that have been shown to interfere with evolution at the level of the subpopulation. Our earlier work (Roulin et al 2013), however, indicated regional adaptation of resting-stage production in *D. magna* on a continental scale. In the light of the current study, it might as well have reflected adaptation at metapopulation or regional levels, rather than at the population level per se.

**DATA ARCHIVING**

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