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1 **Pathogen-induced hatching and population-specific life-history response to**
2 **water-borne cues in brown trout (*Salmo trutta*)**

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17
18 **Abstract**

19 Hatching is an important niche shift, and embryos in a wide range of taxa can either
20 accelerate or delay this life-history switch in order to avoid stage-specific risks. Such behavior
21 can occur in response to stress itself and to chemical cues that allow anticipation of stress. We
22 studied the genetic organization of this phenotypic plasticity and tested whether there are
23 differences among populations and across environments in order to learn more about the
24 evolutionary potential of stress-induced hatching. As a study species we chose the brown
25 trout (*Salmo trutta*; Salmonidae). Gametes were collected from five natural populations
26 (within one river network) and used for full-factorial *in vitro* fertilizations. The resulting
27 embryos were either directly infected with *Pseudomonas fluorescens* or were exposed to
28 water-borne cues from *P. fluorescens*-infected conspecifics. We found that direct inoculation
29 with *P. fluorescens* increased embryonic mortality and induced hatching in all host
30 populations. Exposure to water-borne cues revealed population-specific responses. We found
31 significant additive genetic variation for hatching time, and genetic variation in trait plasticity.
32 In conclusion, hatching is induced in response to infection and can be affected by water-borne
33 cues of infection, but populations and families differ in their reaction to the latter.

34
35 **Keywords:**

36 Additive genetic variation, fish embryo, induced hatching, niche shift, phenotypic plasticity,
37 reaction norm, salmonid

40 **Introduction**

41 The timing of life-history transitions and niche shifts is expected to be influenced by the risk-
42 benefit ratio before and after the change (Werner and Gilliam 1984; Werner 1986; Rowe and
43 Ludwig 1991). As such risk-benefit ratios often vary according to environmental conditions,
44 some degree of phenotypic plasticity is expected (Gomez-Mestre et al. 2008; Reed et al.
45 2011). Hatching represents such a life-history transition and niche shift, and plasticity in this
46 trait has been observed in a number of taxa (Warkentin 2007; Warkentin 2011). Induced
47 hatching is often favored in response to egg-specific stressors (e.g. predators, pathogens)
48 (Warkentin et al. 2001; Wedekind 2002; Moreira and Barata 2005; Vonesh 2005; Gomez-
49 Mestre et al. 2006; Touchon et al. 2006), and conversely, delayed hatching is preferred when
50 there is increased risk of larval mortality (Sih and Moore 1993; Moore et al. 1996; Martin
51 1999; Laurila et al. 2002; Schalk et al. 2002).

52 Notably, the physical presence of a threat is not always required to induce a plastic
53 response in hatching time. Cues from both predators (Moore et al. 1996; Smith and Fortune
54 2009; Miner et al. 2010) and pathogens (Kiesecker et al. 1999; Wedekind 2002) have been
55 observed to alter hatching time. These cues can be quite specific, allowing animals to
56 distinguish between predators and non-predators, and even predator diets (Chivers et al. 2001;
57 Kusch and Chivers 2004). Induced responses can equally be elicited from injured or infected
58 conspecifics (Kiesecker et al. 1999; Chivers et al. 2001; Wedekind 2002; Miner et al. 2010).
59 The ability to respond to a threat before physical contact is not only practical for slower
60 hatching species (e.g. that require proteolytic cleavage of the egg membrane; Anderson and
61 Brown 2009), but is particularly well suited for pathogen challenge as it decreases infection
62 risk.

63 The ability for such inducible defenses to evolve in a population resides in the
64 persistence of genetic variation in trait plasticity (reaction norms) (Pigliucci 2001); however,
65 the genetic organization of a trait can change according to environmental condition, and not
66 necessarily in a consistent direction. For example, genetic variation has been shown
67 repeatedly to increase from favorable to unfavorable conditions (Hoffmann and Merilä 1999;
68 Agrawal et al. 2002; Relyea 2005; Kraft et al. 2006), while other studies reported either a
69 decrease (Merilä 1997; Laugen et al. 2005) or no change in heritable variation with
70 increasingly stressing conditions (Merilä and Fry 1998; Pakkasmaa et al. 2003; Merilä et al.
71 2004). Taken together, these studies suggest that the expression of genetic variation in a trait
72 is not only trait dependent but can also be reliant on the stressor. To add an additional level of
73 complexity, the amount of genetic variation in a trait can also vary among populations, due to
74 genetic drift and/or local adaptation (Einum and Fleming 2000; Gomez-Mestre and Warkentin
75 2007). Indeed, in the case of salmonids, life histories are typically finely tuned to local
76 environmental conditions (Crozier et al. 2008). Consequently, differences in micro-ecological
77 conditions often lead to population-specific reaction norms in early life history traits.

78 While much work has been conducted to characterize patterns of genetic variation in
79 plastic traits at more advanced developmental stages, few have done so during the egg stage
80 (Gomez-Mestre et al. 2008; Jensen et al. 2008). To our knowledge, no study to date has
81 assessed genetic variation for induced hatching in response to water borne cues of infection.
82 Here, we performed full-factorial *in vitro* fertilizations within five populations of brown trout
83 (*Salmo trutta*), and infected resulting embryos with the opportunistic fish pathogen,
84 *Pseudomonas fluorescens* (Austin and Austin 2007). As in Wedekind (2002), water-borne
85 cues from acutely stressed embryos were then taken and, after sterile filtration, applied to
86 conspecifics. By virtue of our experimental design, we were able to observe whether (i)
87 brown trout embryos responded to water-borne cues, (ii) determine if populations differed in
88 their response, (iii) estimate the amount of additive genetic variation for induced or delayed
89 hatching, (iv) and assess whether these patterns differed by population.

90 **Material and Methods**

91 Fertilization protocol and embryo rearing

92 Adult male and female brown trout were caught by electro-fishing from the river network
93 between the cities of Bern and Thun; Switzerland, i.e. the river Aare (from 7°34'16.67"E/
94 46°49'09.58"N to 7°26'46.78"E / 46°56'39.00"N) and four of its tributaries (Kiese:
95 7°37'11.29" E / 46°50'55.85" N; Gürbe: 7°30'4.19" / 46°52'59.37"; Giesse: 7°32'44.00" /
96 46°53'05.33"; Amletenbach: 7°34'04.73" / 46°47'05.95") during the breeding season in
97 October to November 2009. Pairwise comparisons between these populations not only
98 showed significant genetic differentiation but also important morphological differences
99 (Stelkens et al. 2012). After capture, adults were kept at the cantonal hatchery until the start of
100 the experiment. Four females and six males from each population were haphazardly chosen,
101 anaesthetized (Tricaine mesylate MS-222), measured (total length), and stripped of their
102 gametes. These gametes were used for *in vitro* fertilizations (following methods described in
103 Jacob et al. 2007). Crossings were performed within populations in a full-factorial breeding
104 design, i.e. for each population all possible crosses of four females and six males (North
105 Carolina II; Lynch and Walsh 1998) yielding in total 120 sib-groups (24 per population).
106 Water used for fertilizations and thereafter was chemically standardized (OECD 1992),
107 aerated, and cooled to 6.5°C before use.

108 After water hardening, eggs were transported to a climate chamber (6.5°C) where they
109 were washed (as described in von Siebenthal et al. 2009). Eggs were then distributed singly to
110 24-well plates (Falcon, Becton Dickinson; 2ml water per well) in a block-wise design, such
111 that a set of five plates contained all types of siblings. They were examined 54 days post-
112 fertilization on a light table (Hama professional, LP 555) and with a stereo zoom microscope
113 (Olympus SZX9) to assess fertilization success.

114 Isolation of *Pseudomonas fluorescens*, identification, and infection protocol

115 To facilitate the collection of a pathogenic strain of the gram-negative bacterium *P.*
116 *fluorescens*, brown trout eggs from various origins were left in Petri dishes and incubated
117 until signs of infections could be observed. A plate was randomly selected (eggs originated
118 from Amletenbach), and 100µl of water was streaked onto a non-selective tryptic soy agar
119 media (TSA) (Sigma Aldrich) plate and incubated at 22°C for 48 hours. Non-selective media
120 is preferred for single colony selection as it allows for identification of other bacterial
121 contaminants (Holt et al.1994). A fluorescent colony was selected and restreaked onto a TSA
122 plate, which was allowed to incubate for 24 hours. This procedure was repeated twice to
123 ensure colony purity (Sambali and Mehrotra 2009). For identification, DNA was isolated
124 from the colony using the GenElute™ Bacterial Genomic DNA Kit, according to the
125 manufacturer's instructions (Sigma-Aldrich). A PCR was performed using a *P. fluorescens*-
126 specific primer set: 16SPSEfluF (5'-TGCATTCAAACACTGACTG-3') and 16SPSER
127 (5'AATCACA-CCGTGGTAACCG-3') (Sparpellini 2004). *P. fluorescens* strain ATCC
128 17400 was used as the positive control. All PCR reactions were performed in a total volume
129 of 50 µl, and contained: ~50-100ng bacterial genomic DNA, 5 µl of 10X PCR buffer, 200 µM
130 of each dNTP, 2 mM of MgCl₂, 0.5 µM of each primer, and 0.5 U of Taq polymerase
131 (Qiagen). The thermal profile was modified from Scarpellini et al. (2004): 2 min at 94°C; 5
132 cycles of 94 °C for 45s, 55 °C for 1 min, 72 °C for 2 min; 35 cycles of 92 °C for 45s, 60 °C for
133 45s, 72 °C for 2 min; final extension of 72 °C for 2 min; and final cooling at 12 °C. Following
134 the PCR, the amplified products were purified with the Wizard® SV Gel and PCR Clean-Up
135 System (Invitrogen) and sequenced in the forward and reverse directions with
136 16SPSEfluF/16SPSER on a ABI Prism 3100 genetic analyzer (Applied Biosystems).

137 To prepare the bacterial inoculum, 200 ml of tryptic soy broth (TSB) was inoculated
138 with a single colony of *P. fluorescens*. The flask was then placed on a shaker (200rpm) for 36
139

140 hours at 22°C, until reaching exponential growth phase. The contents were transferred to 50
141 ml conicals and centrifuged for 15 minutes at 4000 rpm. Pellets were washed twice with
142 sterile standardized water and pooled. Bacterial cells were counted with a Helber
143 hemocytometer using a phase contrast microscope (400x). The suspension was diluted such
144 that an inoculation with 100 µl/well would result in a concentration of 6.0×10^8 cells/ml.

145 Eight of the twenty replicates per sibgroup were treated with *P. fluorescens* 54 days
146 post-fertilization. The remaining 12 replicates received only standardized water. Nine days
147 later, 100µl of water were taken from each treated well, pooled, and sterile filtered (0.2µm) to
148 produce the water-borne cue (WBC). Eight of the 12 non-infected embryos per sibgroup were
149 then exposed to 100µl of WBC. The remaining four embryos served as controls and were
150 sham-treated with sterile-filtered standardized water. Following treatment, embryos were
151 monitored daily and mortality and hatching date were recorded.

152 153 Statistical analyses

154 Analyses were performed with Jmp7.0 (JMP 1989-2007) and R (R Development Core Team
155 2006) using the lme4 package for linear and logistic mixed effect model analyses (Bates and
156 Sarkar 2007). Eggs that were not eyed on day 54 post fertilization were assumed to be non-
157 fertilized and hence excluded from all further analyses. Survival was analyzed as a binomial
158 response variable in generalized linear mixed models (GLMM), and hatching time as a
159 continuous variable in linear mixed models (LMM). Treatment was considered as fixed effect,
160 and population, dam, sire, treatment x population, treatment x dam, and treatment x sire
161 interactions as random effects. Starting with a reference model that included treatment,
162 population, dam, and sire, we added or removed one effect at a time and compared the
163 reference and the changed model with likelihood ratio tests (LRT) using restricted maximum
164 likelihood (REML) (Zuur et al. 2009). Sire x dam interactions were not included in the
165 models to avoid low sample sizes in some experimental cells.

166 Because infection with *P. fluorescens* and inoculation with WBC were performed at
167 two different time points during embryo development, and reactions to stressors may be
168 conditional to developmental stage (Johnson et al. 2001; Schotthoefer et al. 2003; Sollid et al.
169 2003; E. S. Clark et al., unpubl. data), infected embryos were not directly compared to WBC-
170 exposed embryos. Instead, both treatments were compared to the controls only. Moreover,
171 because embryo survival was low in the infection treatment, survival but not hatching date
172 was analyzed in the corresponding mixed effect models. Analogously, because survival
173 turned out to be close to 100% in the WBC treatment (see Results), hatching date but not
174 survival was analyzed in the corresponding mixed effect models.

175 Variance components for hatching time were extracted from mixed models using
176 REML. With a full-factorial breeding design, assuming that epistasis is negligible, additive
177 genetic variance (V_A) is estimated as four times the sire variance (V_{SIRE}) (Lynch and Walsh
178 1998). Maternal environmental effects (V_M), i.e. the part of dam variance (V_{DAM}) not
179 explained by additive genetic effects, was calculated by subtracting V_{SIRE} from V_{DAM} .
180 Residual variance (V_{RES}) includes environmental variance as well as $\frac{1}{2} V_A$ and $\frac{3}{4}$ dominance
181 variance (V_D) (Kearsey and Pooni 1996). Narrow sense heritability was estimated as V_A
182 divided by total variance, i.e V_{DAM} , V_{SIRE} and V_{RES} , as in Lynch and Walsh (1998). Standard
183 errors of variance components and heritabilities were calculated using a bootstrap procedure,
184 taking the standard deviation of 1000 estimates produced by reshuffled hatching dates
185 separately per population and treatment in R. To facilitate comparisons with other studies, we
186 also report the coefficients of additive genetic variation (CVA) (Houle 1992). Variance
187 components and heritability were not estimated for the binominal response variable survival,
188 because mortalities turned out to be either very low (controls, WBC) or very high (infected)
189 rendering heritability estimates from count data estimates spurious. Genetic correlations for

190 hatching time across two environments were estimated using Pearson's product moment
191 correlations for sire sib-group means (Roff 1997; Relyea 2005; Gomez-Mestre et al. 2008).

192

193 **Results**

194 Embryo survival

195 Exposure to *P. fluorescens* significantly decreased mean survival until hatching from $98.8 \pm$
196 0.5% (SE) in the control to $11.6 \pm 1.1\%$ in infected eggs (Table 1). Populations did not differ
197 significantly in average embryo viability or susceptibility to infection (Table 1). While sire x
198 treatment or dam x treatment interactions were not significant (Table 1), there were
199 significant overall dam and sire effects in offspring viability (Table 1). Survival in the WBC
200 treatment was on average $97.3\% \pm 1.0\%$ (SE) and not significantly different from the controls
201 (GLMM: $z = -1.58$, $p = 0.12$).

202

203 Treatment and population effects on hatching time

204 Embryos treated with *P. fluorescens* hatched significantly earlier than controls (comparing the
205 hatching times of the first hatchling per sibgroup: median difference = 60 hours, Wilcoxon
206 signed rank test, $V = 561.5$, $p = 0.006$; mean hatching time per sibgroup: median difference =
207 57 hours, $V = 587$, $p = 0.002$; Fig. 1). This median difference in the timing of first hatching
208 per sibgroup corresponded to a median hatching acceleration of 18% since exposure to *P.*
209 *fluorescens* and did not significantly among the populations (Kruskal-Wallis $\chi^2 = 4.7$, d.f. = 4,
210 $p = 0.32$). Reaction to WBC treatment varied among populations (Table 1; Fig. 1). Treatment
211 with WBC induced hatching in the Gürbe population, delayed hatching in Giese and
212 Amletenbach, and appeared to have no significant overall effect in Kiese and Aare (Fig. 1).

213 We found significant overall dam and sire effects on hatching time (the main effects in
214 Table 1). We also found significant genetic variation for hatching plasticity (the sire x
215 treatment and dam x treatment interaction in Table 1). In the control, sire effects accounted
216 for a significant part of the variance in two populations, i.e. Aare (LRT: $\chi^2 = 7.85$, $p = 0.005$)
217 and Gürbe ($\chi^2 = 17.53$, $p < 0.0001$), but were not significant in Kiese ($\chi^2 = 0.08$, $p = 0.78$),
218 Giese ($\chi^2 = 0.07$, $p = 0.80$) or Amletenbach ($\chi^2 = 0.00$, $p = 1.00$) (Table 2). However in the
219 WBC treatment, sire effects were significant in four out of five populations, i.e. Aare ($\chi^2 =$
220 13.97 , $p = 0.0002$), Gürbe ($\chi^2 = 19.30$, $p < 0.0001$), Giese ($\chi^2 = 56.66$, $p < 0.0001$) and
221 Amletenbach ($\chi^2 = 13.93$, $p = 0.0002$), but not Kiese ($\chi^2 = 0.56$, $p = 0.45$) (Table 2).

222

223 Cross-environmental trait correlations

224 As populations differed significantly in hatching response to treatments (Table 1), cross-
225 environment genetic correlations between control and WBC were calculated separately for
226 each population. For the Kiese and Giese, there is no correlation in hatching time among the
227 two environments ($r = -0.01$, $p = 0.98$; and $r = 0.66$, $p = 0.15$ respectively). However,
228 hatching time was positively correlated among the two environments in Aare ($r = 0.82$, $p =$
229 0.04), Gürbe ($r = 0.89$, $p = 0.02$) and Amletenbach ($r = 0.86$, $p = 0.03$).

230

231 **Discussion**

232 In whitefish (*Coregonus* sp.), i.e. in another salmonid genus, exposure to *P. fluorescens* has
233 been shown to cause significant embryonic mortality (Wedekind et al. 2004; von Siebenthal
234 et al. 2009), as well as to induce hatching (Wedekind 2002). Brown trout embryos differ in
235 many respects from whitefish embryos, including mean size, various characteristics of their
236 typical habitat, and the behavior of freshly hatched alevins (Wedekind and Müller 2005). We
237 nevertheless found that treatment with *P. fluorescens* increased mortality and also prompted
238 precocious hatching. Sensitivity to *P. fluorescens* and the host's life-history response
239 therefore appears to extend to brown trout embryos. This allowed us to compare populations

240 that live in different habitats (but still within the same catchment area) and for which Stelkens
241 et al. (2012) found significant morphological and genetic differentiation despite the rather
242 small geographic scale. Some of the previously observed morphological differentiation within
243 this catchment area could even be linked to habitat characteristics (Stelkens et al. 2012),
244 consistent with local adaptation.

245 Although experimental infection with *P. fluorescens* induced mortality and precocious
246 hatching in all study populations, we found that the populations varied significantly in their
247 hatching response to water-borne cues from infected embryos, i.e. we found significant
248 differences in the reaction norms. Such variation at the level of the population could
249 potentially indicate differential selection pressures (Laurila et al. 2002; Jensen et al. 2008). As
250 the microbial community composition can differ vastly between streams (Evans and Neff
251 2009), it is possible that our five study populations would typically be exposed to different
252 pathogen communities. However, differences between populations could also result, for
253 example, from genetic drift. We further found significant dam and sire by treatment
254 interactions, i.e. additive genetic variation for hatching plasticity. Such variability in reaction
255 norms could indicate a potential for adaptation in response to environmental changes
256 (Hutchings 2011). However, as dams and sires were nested in population, family effects
257 cannot fully be disentangled from potential population differences.

258 The study populations not only differed in their reaction to water-borne cues but also
259 in the genetic organization of hatching time. While our overall analysis showed significant
260 amounts of additive genetic and maternal environment variation in hatching time across
261 treatments, closer examination within populations revealed more variable patterns. Certain
262 populations seemed to have little genetic variation for hatching time under benign conditions.
263 This finding would meet traditional expectations for life-history traits, which anticipate
264 reduced genetic variation as a consequence of directional selection (Mousseau and Roff 1987)
265 or stabilizing selection (Fisher 1930). However, significant additive genetic variance for the
266 trait was found in some other populations, conforming with studies which have shown
267 considerable heritable variation in fitness-related traits (Houle 1992; Merilä and Sheldon
268 1999).

269 The water-borne cues we used likely consisted of a complex mixture of chemicals of
270 both pathogen and embryo origin. On the one hand, these chemicals could mainly convey an
271 imminent danger to embryos, as it did in Kiesecker et al. (1999) and Wedekind (2002). On the
272 other hand, they may also relay other kinds of information that may be important in other than
273 the host-parasite context, e.g. they could reveal that conspecifics were in the environment.
274 The presence or absence of conspecifics has been shown to induce or delay hatching time in
275 other species (Miner et al. 2010). It therefore remains unclear what aspect of a potential
276 multiple signal the embryos reacted to in our experiment. Additionally, it remains to be seen
277 how the difference in the timing of exposure to pathogen versus the water-borne cues may
278 have impacted the behavior response to treatment. Nevertheless, the population differences
279 we observed suggests that hatching time in brown trout is not only dependent on
280 environmental factors, but that these environmental factors are differently weighted by
281 embryos of different origins.

282 Despite significant overall dam and sire effects on embryo mortality, we found no
283 significant population x treatment effects on mortality, indicating a more or less uniform
284 susceptibility to infection. We further found no sire or dam by treatment interactions,
285 indicating a lack of genetic variability in survival reaction norms. Lack of variation in
286 reaction norms at both the family and population level could have important implications on
287 the species' ability to cope with infectious disease—a stressor which is anticipated to increase
288 in occurrence due to anthropogenic activities (Dewitt et al. 1998; Harvell 1999; Dobson and
289 Fougopoulos 2001). However, significant gene by environment interactions on larval survival

290 could be found in other salmonids, e.g. in the Chinook salmon (*Oncorhynchus tshawytscha*)
291 (Evans et al. 2010) or in whitefish (*Coregonus* sp.) (von Siebenthal et al. 2009), and even in
292 other populations of brown trout (Wedekind et al. 2008; Jacob et al. 2010). Genetic variability
293 in reaction norms may therefore depend on the kind of stressor, the developmental stage, and
294 the species or population in question.

295 As with pathogen susceptibility, we found significant additive genetic variation for
296 hatching time. In addition, we found genetic variation in hatching plasticity after exposing
297 embryos to water-borne cues of infection. We further observed that hatching time was
298 correlated across control and WBC treatments in the majority of the populations, indicating
299 that trait expression is mediated by the same loci in different environments (Via 1984). Trait
300 means, therefore, may not be able to evolve independently, which places a constraint on the
301 evolutionary potential of the reaction norm (Pigliucci 2001).

302

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311

312 **Ethical standards**

313 Permissions for handling embryos were granted by the local authority (i.e. the Fishery
314 Inspectorate of the Bern canton). The manipulations of the adults were part of the yearly
315 hatchery program of the Bern canton. Experimental manipulations on embryos were
316 performed prior to yolk sac absorption. All manipulations comply with the current law of the
317 country in which they were performed (Switzerland). The authors declare that they have no
318 conflict of interest.

319

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479

480 Table 1 Likelihood ratio tests on mixed model logistic regressions on (a) embryo survival,
 481 and (b) hatching time. Two treatments are compared each, i.e. control vs. infected and control
 482 vs. WBC (see main text). The Akaike information criterion (AIC) indicates the goodness of
 483 fit of a model. P-values were obtained from comparisons between the respective model and
 484 the reference model (in bold)

| Model | Effect tested | DF | AIC | X ² | P |
|---------------------|---------------------|----|--------|----------------|---------|
| (a) Survival | | | | | |
| Control vs Infected | | | | | |
| T+P+D+S | | 5 | 520.9 | | |
| T+T P+D+S | Treat. x Population | 7 | 524.5 | 0.4 | 0.81 |
| T+P+T D+T S | Treat. x Dam | 7 | 523.3 | 1.6 | 0.44 |
| T+P+D+T S | Treat. x Sire | 7 | 520.4 | 4.5 | 0.10 |
| T+D+S | Population | 4 | 518.9 | 0.0 | 1.00 |
| T+P+S | Dam | 4 | 580.3 | 61.3 | <0.0001 |
| T+P+D | Sire | 4 | 608.8 | 89.9 | <0.0001 |
| P+D+S | Treatment | 4 | 1754.2 | 1235.3 | <0.0001 |
| (b) Hatching time | | | | | |
| Control vs WBC | | | | | |
| T+P+D+S | | 6 | 9423.6 | | |
| T+T P+D+S | Treat. x Population | 8 | 9371.9 | 55.6 | <0.0001 |
| T+P+T D+S | Treat. x Dam | 8 | 9393.2 | 34.4 | <0.0001 |
| T+P+D+T S | Treat. x Sire | 8 | 9389.1 | 38.4 | <0.0001 |
| T+D+S | Population | 5 | 9424.5 | 2.9 | 0.09 |
| T+P+S | Dam | 5 | 9835.2 | 413.6 | <0.0001 |
| T+P+D | Sire | 5 | 9544.5 | 122.9 | <0.0001 |
| P+D+S | Treatment | 5 | 9422.0 | 0.4 | 0.52 |

485

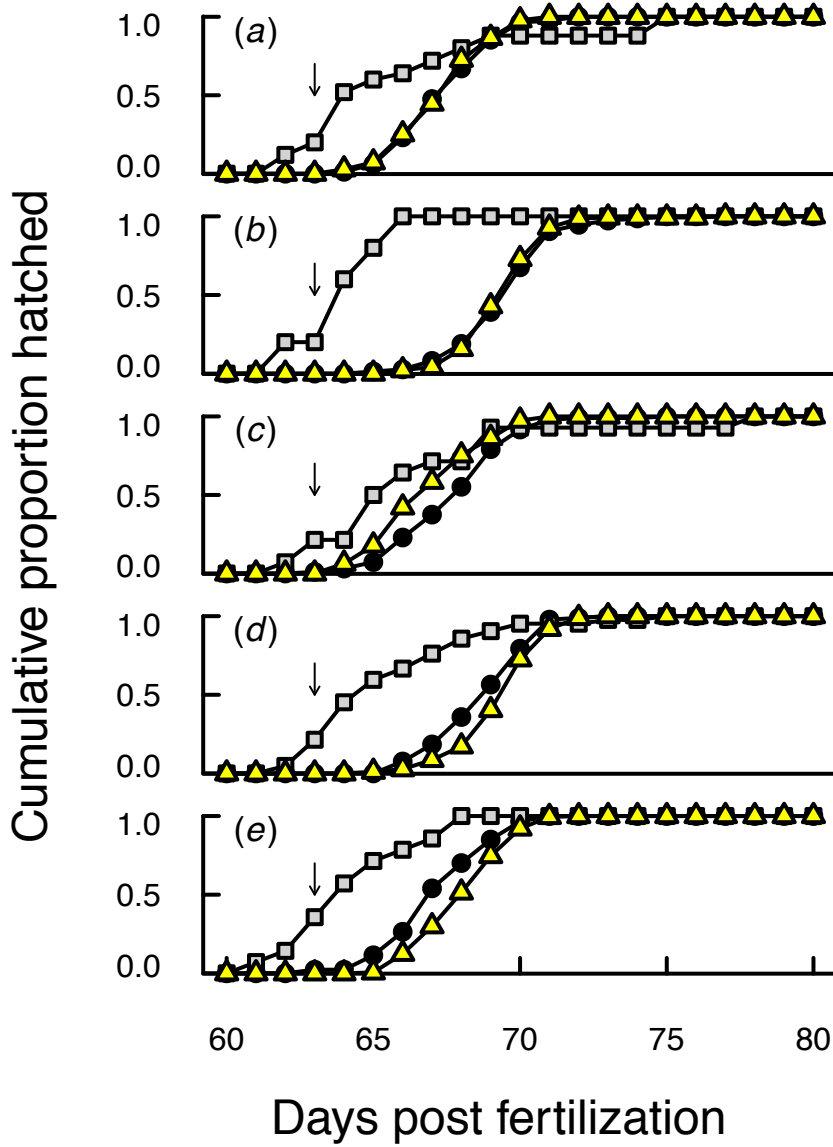
486 Table 2 Within-environment variance components (V_A : additive genetic variance, V_M :
 487 maternal effect variance, V_{RES} : residual variance), narrow sense heritability estimate (h^2) and
 488 coefficients of additive genetic variation (CV_A) for hatching time in the control and the WBC
 489 treatment. Numbers in parentheses indicate standard errors; asterisks indicate whether males
 490 explain a significant part of the variance (see main text)
 491

| Population | V_A | V_M | V_{RES} | h^2 | CV_A |
|--------------------|-----------------|-------------|-------------|-------------|-------------|
| Hatching time | | | | | |
| (a) Control | | | | | |
| Kiese | 6.2 (14.2) | 40.9 (5.0) | 80.9 (16.9) | 0.05 (0.12) | 0.56 (0.52) |
| Aare | 108.8 (17.3)** | -2.7 (6.1) | 85.4 (24.8) | 0.79 (0.14) | 2.30 (0.56) |
| Gürbe | 132.7 (19.2)*** | 31.8 (6.3) | 81.4 (26.0) | 0.74 (0.12) | 2.60 (0.60) |
| Giesse | 4.5 (13.4) | 31.0 (4.5) | 82.6 (13.2) | 0.04 (0.12) | 0.47 (0.49) |
| Amletenbach | 0.0 (15.7) | 38.5 (5.2) | 96.6 (17.4) | 0.00 (0.12) | 0.00 (0.54) |
| (b) WBC | | | | | |
| Kiese | 4.7 (6.3) | 49.1 (2.1) | 63.0 (9.8) | 0.04 (0.06) | 0.49 (0.34) |
| Aare | 67.2 (6.6)*** | -13.1 (2.3) | 70.7 (17.8) | 0.74 (0.08) | 1.81 (0.35) |
| Gürbe | 63.0 (7.9)*** | 47.6 (2.6) | 78.0 (11.2) | 0.40 (0.06) | 1.82 (0.39) |
| Giesse | 107.4 (5.1)*** | -2.6 (1.7) | 47.3 (10.4) | 1.09 (0.06) | 2.29 (0.30) |
| Amletenbach | 28.2 (5.7)*** | 48.9 (1.9) | 45.6 (8.1) | 0.26 (0.06) | 1.20 (0.33) |

492 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

493

494 **Fig. 1** Cumulative proportion hatched per population (a: Kiese ; b: Aare; c: Gürbe; d: Giesse;
495 e: Amletenbach) and treatment (control: round symbols; *P. fluorescens*-treated: squares;
496 WBC-treated: triangles). The arrows indicate the timing of the WBC treatment
497



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