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1 **Can we predict the effects of multiple stressors on insects in a changing climate?**

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11

12 Abstract

13 The responses of insects to climate change will depend on their responses to abiotic and
14 biotic stressors in combination. We surveyed the literature, and although synergistic stressor
15 interactions appear common among insects, the thin taxonomic spread of existing data means
16 that more multi-stressor studies and new approaches are needed. We need to move beyond
17 descriptions of the effects of multiple stressors to a mechanistic, predictive understanding.
18 Further, we must identify which stressor interactions, and species' responses to them, are
19 sufficiently generalizable (i.e. most or all species respond similarly to the same stressor
20 combination), and thus predictable (for new combinations of stressors, or stressors acting via
21 known mechanisms). We discuss experimental approaches that could facilitate this shift
22 towards predictive understanding.

23

24 **Introduction**

25 Insects and other organisms must simultaneously respond to various, natural abiotic and
26 biotic stressors, as well as to an increasing array of novel anthropogenic environmental
27 stressors. It is often difficult to distinguish the origin of stress because natural abiotic or biotic
28 stressors can be exacerbated by anthropogenic influences. Some of the most important
29 human-induced or -accelerated environmental stressors include climate change, habitat
30 fragmentation, chemical pollution and introduction of invasive species [1,2]. Different
31 stressors will interact in various possible ways under climate change, and the responses and
32 resistance of organisms to climate change will depend on their responses to combinations of
33 stressors, which may or may not reflect responses to single stressors in isolation.

34

35 Multiple stressors have been studied systematically in aquatic ecosystems for non-insect
36 organisms, especially in ecotoxicology [3–6] [7^{*}], where it appears that synergistic
37 interactions (resulting in a greater-than-expected impact) among stressors may be the norm.
38 Here we show that multiple stressor studies on insects are still relatively rare, and discuss best
39 practices in experimental design for multiple stressor studies that aim to characterise
40 responses and identify underlying mechanisms, with an overall goal of predicting *a priori* the
41 outcomes of interacting stressors.

42

43 **What do we study when we study multiple stressors?**

44 We define a ‘stressor’ as any environmental or biotic factor with the potential to disrupt
45 homeostasis, performance or fitness [8]. The consequences of exposure to multiple stressors
46 are usually separated by ecologists and ecotoxicologists into additive and non-additive
47 (synergistic or antagonistic) effects [3,9–12]. In this review, we use the terms “synergism”
48 and “antagonism” as a convenient way to indicate stressor interactions that result in greater or

49 lesser effects than the additive sum of effects produced by the stressors acting in isolation,
50 respectively [see 5]. A range of statistical models have been used to identify non-additive
51 effects, and, as a consequence, the strength and basis of conclusions may depend on the
52 experimental context [9,12,13]. Some authors advocate using ‘synergism’ and ‘antagonism’
53 only to refer to effects detected by means of additive ANOVA models in a full-factorial
54 design [3,9,10,13,14]. By contrast, in some disciplines, for example toxicology, the mode of
55 actions of multiple toxicants (stressors) are always assumed to be different, leading to
56 multiplicative (rather than additive) effects, which therefore requires appropriate statistical
57 analysis and terminology [9,15,16].

58

59 We informally surveyed the Anglophone insect literature (see supplementary material for
60 details of the studies we identified). To narrow our criteria we focused on studies that
61 manipulated different classes of stressors (e.g. temperature and pathogens) in full-factorial
62 designs that allow estimation of non-additive results. A full-factorial two-stressor study
63 would thus include four treatments; (1) neither treatment (control), (2) stressor A alone, (3)
64 stressor B alone, and (4) both stressors, A and B, in combination. Despite the prevalence of
65 multiple, interacting, stressors in nature, most insect studies explore effects of one stressor in
66 isolation. At least 210 studies applying two stressors were not full-factorial in study design
67 (e.g. lacking a stressor-free control or an individual stressor treatment). Some of those studies
68 (e.g. those exploring interactions between temperature and pathogens and temperature and
69 atmospheric gases) use an additional stressor to enhance the impact of a stressor of primary
70 interest [17^{*}], and do not use a full-factorial experimental design. Such studies cannot predict
71 the range of non-additive responses and do not provide material for future meta-analyses on
72 stressor interactions. We found 133 full-factorial studies (listed in Supplementary Word file),
73 covering 24 stressor pairs (Table S1; Table S2). Fewer than ten studies included three-

74 stressor combinations, and we found none including more than three stressors. The two-
75 stressor studies were spread across 12 insect orders, 51 families and 100 species (Fig. S1).
76 This coverage of insects is still low for making far-reaching generalisations, especially when
77 the stressor outcomes are split among many stressor pairs.

78

79 The choice of endpoints in multiple stressor studies can substantially influence the
80 conclusions [18^{*},19]. Many studies used mortality as an endpoint, for example those that
81 explored the combined lethality of chemical and pathogen/temperature exposures. Although
82 these studies can reveal non-additive stressor interactions, in our opinion they cannot reveal
83 their underlying mechanisms. Many two-stressor studies used sub-lethal, fitness-related,
84 endpoints, such as growth or fecundity (Fig. S1), which likely reflect diversion of energy to
85 repair stress damage and re-establish homeostasis [2,20,21]. We suggest that fitness-related
86 endpoints can shed light on underlying mechanisms of ecologically-relevant interactions
87 among stressors.

88

89 Although the studies we included were full-factorial in design, many were unable to directly
90 identify synergy or antagonism because of the selected statistical approach (e.g. comparing
91 combined effects only to the univariate effects of one stressor, but not both). Thus, the results
92 can provide only the presumption of non-additive interactions between stressors (Fig. S1;
93 Table S2). However, studies incorporating chemical stressors (especially chemical-
94 temperature and chemical-pathogen interactions) more routinely used full-factorial statistical
95 models. These studies reveal that chemicals, such as pesticides, often interact synergistically
96 with temperature or pathogen stress. For example, in the blue-tailed damselfly *Ischnura*
97 *elegans*, acetylcholinesterase inhibition by the pesticide chlorpyrifos was synergistically
98 magnified when combined with heat stress [18^{*}]. This possible predominance of synergistic

99 interactions is consistent with observations for other animals [3,5]. We argue that the thin
100 taxonomic spread of existing data makes the insect literature currently unsuitable for formal
101 meta-analyses, but the accumulating number of primary full-factorial studies will eventually
102 be useful to identify large-scale patterns in non-additive effects among insect species and
103 stressors.

104

105 **Accounting for the multiple scales of multiple stressors**

106 The timing, intensity, duration, frequency and spatial distribution of stressor exposure will
107 vary among stressors and among stressor events, complicating their inclusion when
108 predicting organismal responses to multiple stressors and global change [2,22], particularly
109 when complex systems can give rise to emergent phenomena [23]. Including all possible
110 stressor combinations (and durations, sequences and intensities of stressor exposures) will
111 rapidly become intractable, but the spatio-temporal complexity of multiple stressors can be
112 reduced for experimental purposes by selecting combinations on the basis of their (co-)
113 occurrence dynamics in nature, for example, using the fractional factorial approach espoused
114 by Porter et al. (1984) [24**].

115

116 The intensity (loosely, 'dose') of stressors can determine the outcome of interactions. This is
117 best exemplified in the concept of hormesis, wherein small doses of stress can be beneficial,
118 but large doses damaging [25]. This effect of intensity is prevalent in responses to many
119 kinds of physical, chemical or biological stressors, for example, the heat shock response is
120 induced at a threshold temperature [26], which can then substantially modify responses to
121 other stressors; however, exposure below that threshold will have minimal effect, and
122 exposure to higher temperatures may be lethal. Thus, identifying the minimum 'dose'
123 required to elicit a stress response (or an interaction – which may be a higher or lower dose)

124 must be included in the design of experiments and choice of stressors. One approach to this
125 is to standardise the impact of stressors (the ‘toxic unit approach’ of ecotoxicology) to allow
126 interactions to be explored under conditions where each stressor has similar impact [cf. 27].
127 However, this standardisation may only be ecologically-relevant if the stressors in nature are
128 expected to co-occur at similar intensities.

129

130 The mechanisms underlying the physiological responses to stress can be tightly linked to the
131 spatio-temporal dynamics of co-occurring stressors. For example, the physiological responses
132 to thermal, drought or starvation stress, which often co-occur, are similar in insects [28–30].
133 By contrast, insects may experience other stressors together for the first time under global
134 change; for example, neonicotinoid insecticides and invasive pathogens [31]. Further,
135 changing climates could change the timing and severity of overlapping interacting stressors
136 [28*]. If a change in the sequence of two stressors does not affect the outcome and the result
137 is repeatable in one or several species, or when additional stressors are present, then the
138 responses may have underlying shared mechanisms, and thus mutually-predictable responses,
139 even if they have not previously co-occurred in nature [32,33].

140

141 **Mechanisms underlying insect responses to multiple stressors**

142 Identifying the mechanisms underlying stressor interactions could help us to predict *a priori*
143 the effects of novel combinations of stressors, or to generalise the effects of multiple stressors
144 among taxa [2]. This is especially challenging when our understanding of the mechanistic
145 basis for insects’ responses to even simple single stressors is incomplete. Predictable and
146 generalizable responses to multiple stressors could arise through cross-tolerance – shared
147 mechanisms of stress response that impart protection against multiple stressors once activated
148 – or through cross-talk, whereby signaling responses to the first stress also activate resistance

149 to other stressors [28^{*}]. These underlying processes are generally conceived as adaptive,
150 leading to antagonistic effects of combined stressors, to the benefit of the insect. For
151 example, prior exposure to dehydration improves subsequent tolerance to (i.e. reduces the
152 impact of) cold or heat in the Antarctic midge *Belgica antarctica*, the cross-tolerance
153 facilitated by accumulation of trehalose [29]. In addition, cross-tolerance between low
154 temperature and hypoxia (or other controlled atmospheres), and their underlying
155 physiological and molecular mechanisms have been studied to some extent in insects
156 [17^{*},34–36]. For example, in the false codling moth *Thaumatotibia leucotreta* mild pre-
157 treatments with chilling and hypoxia increased resistance to low temperatures and these
158 responses were correlated with increased membrane fluidity and/or alterations in heat shock
159 protein (HSP70) [36]. The antagonistic effects of cross-tolerance or cross talk are
160 conceptually (and likely mechanistically) related to hormesis [25].

161

162 However, this adaptive framework based on shared responses to stressors predicts
163 antagonistic responses to multiple stressors and thus appears to be at odds with the
164 preponderance of synergistic effects of multiple stressors that we observed in our literature
165 survey (Fig. S1). Synergistic stressor interactions in insects have been most commonly
166 reported for chemical-temperature and chemical-pathogen pairs and the effects of other
167 stressor pairs have been little-studied. Thus, we lack both either the breadth of descriptive
168 data or (for many stressors) the mechanistic understanding of their mode of action necessary
169 to make predictions within this framework. However, mechanism can predict synergistic
170 responses to combined stressors, as in a scarabaeid beetle in which application of an
171 insecticide weakens the immune system, leading to a synergistic interaction when the
172 insecticide is applied in concert with a fungal pathogen [37]. These mechanism-based non-
173 additive interactions can easily yield both synergistic and antagonistic results. For example,

174 cell membrane fluidity can determine cold tolerance in the collembolan *Folsomia candida*, so
175 lipophilic contaminants can either increase or reduce cold tolerance, depending on each
176 contaminants' impact on the phospholipid membrane – a property that can be predicted in
177 advance [38^{*}]. Thus, predicting the impacts of multiple stressors based on mechanism may be
178 primarily hampered by a lack of understanding of the mechanisms underlying the impact of
179 each stressor in isolation.

180

181 **Can we generalize multiple stressor effects to yield predictions?**

182 Currently, the insect literature is dominated by descriptive studies that characterise the
183 responses of a specific taxon to a specific combination of (usually two) stressors. When
184 designed well, these studies can identify non-additive interactions, and hint at underlying
185 mechanisms or pathways shared among stressors. However, the millions of insect species
186 and thousands of stressors mean there are trillions of potential stressor-taxon combinations,
187 so such descriptive studies fall short if we wish to account generally for multiple interacting
188 stressors in our understanding of climate change. To make *a priori* predictions about the
189 consequences of multiple interacting stressors, we first need to determine if the responses to
190 multiple stressors are predictable from an understanding of univariate or bivariate responses,
191 and second, determine whether such predictions are generalizable among taxa. If responses to
192 stressors are predictable, then we can draw larger conclusions about responses to novel
193 combinations of stressors (Fig. 1). Such predictability will likely arise when there are shared
194 mechanisms (or perhaps signaling pathways) underlying responses to those stressors. Thus,
195 univariate studies of single stressors, and the physiological and molecular mechanisms
196 underlying insect responses to them, are essential.

197

198 We term responses to multiple stressors as ‘generalizable’ when most or all species exhibit
199 broadly similar responses to the same stressor combination, but ‘idiosyncratic’ if different
200 species respond differently or in a context-dependent manner (Fig. 2). The shared
201 mechanisms that underlie many stress responses [29,36,39,40], and the associations of at least
202 some stressors over evolutionary time [29] lead us to expect that at least some interactions
203 among stressors will have impacts generalizable to higher taxonomic levels. There are well-
204 established conceptual and analytical tools to assess physiological responses in a
205 phylogenetic context [41,42]. Although these have largely been applied to individual
206 stressors in insects [43–47], we expect that a phylogenetically-cogent approach, for example
207 with *Drosophila* [47^{*}] will yield information on the prevalence of idiosyncratic vs.
208 generalizable responses to multiple stressors.

209

210 An alternative approach is to understand the structure of multiple stressor responses. When
211 exposed to increasing numbers of combinations of stressors, it is possible for the cumulative
212 effects to saturate or accelerate (Fig. 3). A saturation of responses would arise if there are a
213 limited number of possible interaction mechanisms among stressors, such that additional
214 stressors have limited additional impact after some threshold. By contrast, if synergistic
215 interactions combine and become increasingly synergistic with additional stress (or there are
216 emergent properties), the effect of additional stressors may continue to increase. Increasing
217 number of stressors in the green alga *Chlamydomonas reinhardtii* have limited impact on
218 population growth after c. four stressors, suggesting a saturation structure to multiple
219 interacting stressors in this species [48^{**}], and saturation also appears to apply to toxicants
220 (the “funnel hypothesis”) [49^{**}]. However, such experiments have not, to our knowledge,

221 been conducted in insects, and even the few three-stressor studies we identified do not have
222 sufficient stressors to tease these two possible responses apart.

223

224 **Conclusions**

225 Although we know a lot about how insects respond to single stresses, few studies have
226 characterized responses to two stressors in combination, and studies that include three or
227 more stressors are rare. Similarly, although the mechanisms underlying responses to
228 univariate stressors have been explored, we cannot yet connect those mechanisms to the
229 responses to stressors in combination. We suggest that using these data in phylogenetic or
230 multiple stressor frameworks may allow determination of the predictability and
231 generalizability of responses to multiple stressors, and that determining this will improve our
232 ability to incorporate multiple stressors in more general models of global change.

233

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240

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- 243 • of special interest
- 244 •• of outstanding interest

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 393 **hypothesis.** *Ecotoxicol. Environ. Saf.* 1995, **31**:23–28.
- 394 This paper includes a theoretical treatment of the saturation response to multiple toxicants.

395

396

397

398 FIGURE LEGENDS

399

400 **Figure 1**

401 Shared response mechanisms can hypothetically be used to predict responses to novel stressor
402 combinations. Stressor pairs P+Q and P+R share same mechanism (X) resulting in a shared
403 response or outcome (O1). In this example, the new stressor pair T+S also share response
404 mechanism X, so we predict the O1 -response.

405

406 **Figure 2**

407 Responses to interacting stressors can be generalizable or idiosyncratic. This may be evident
408 when responses are compared across a phylogeny (A), where a strong phylogenetic constraint
409 can imply a generalizable response. We speculate that generalizable responses arise when
410 mechanisms are shared (Figure 1), but idiosyncratic, if the same mechanisms yield different
411 responses (B), or if the mechanisms themselves are context-dependent (C).

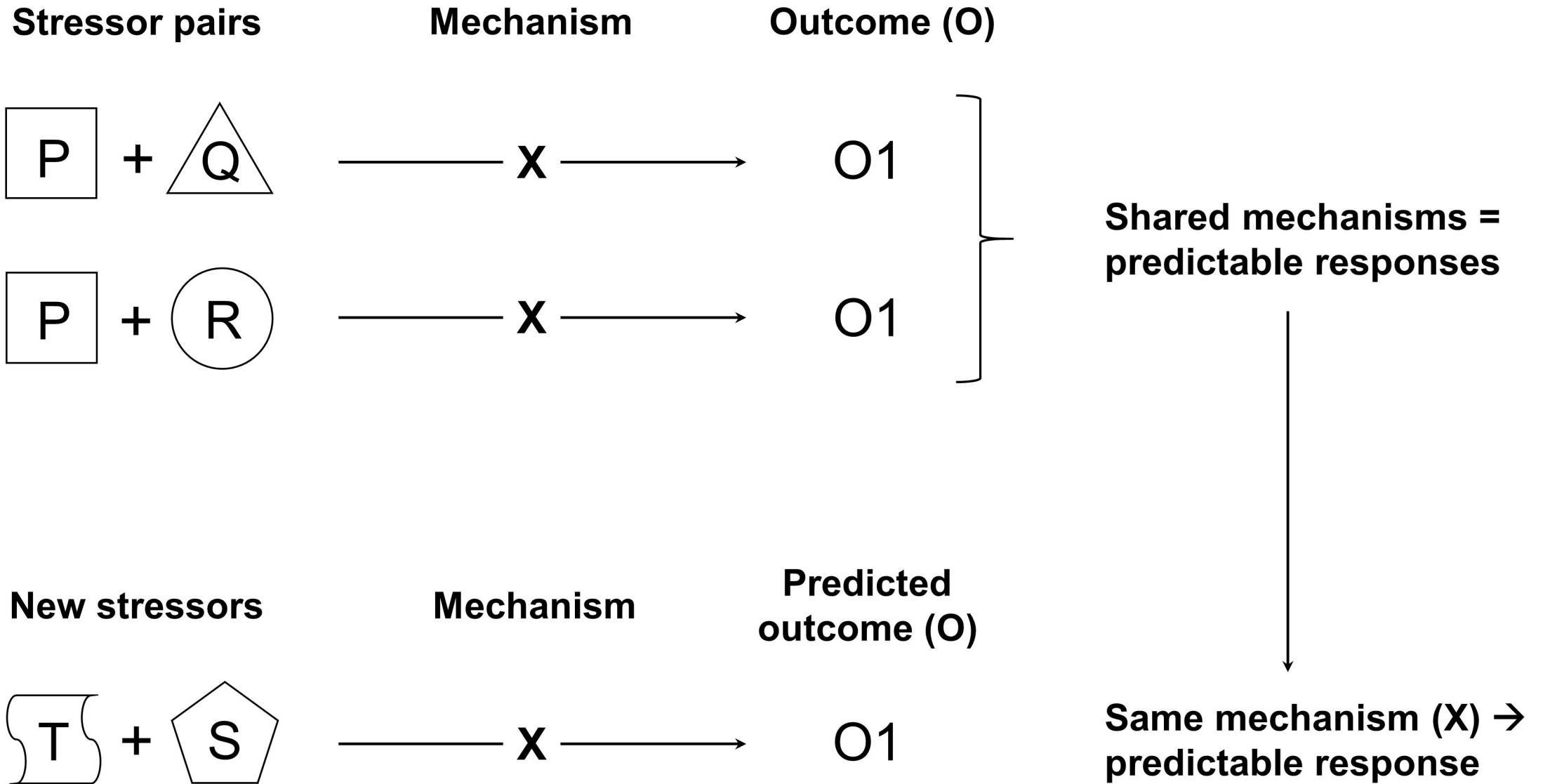
412

413 **Figure 3**

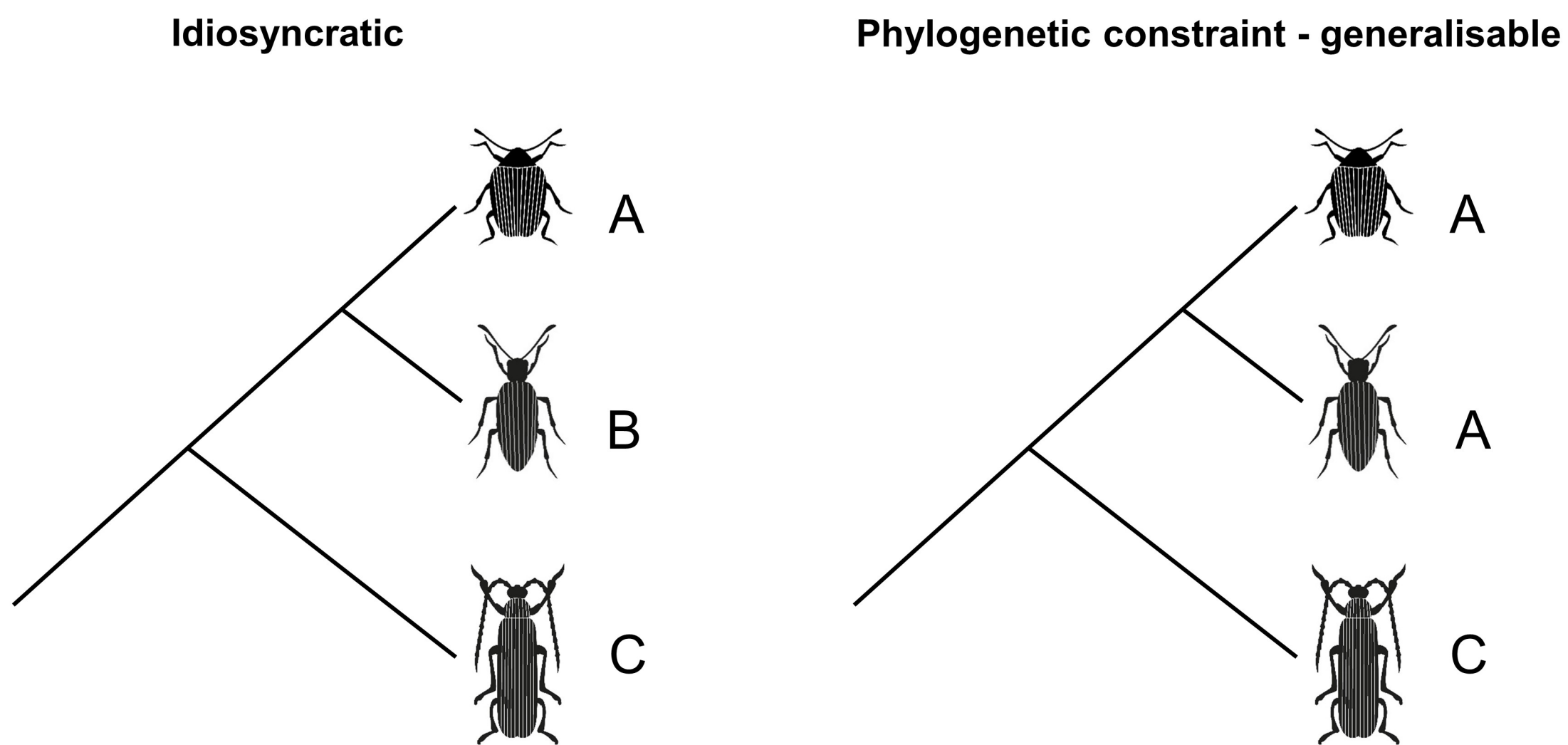
414 When exposed to increasing numbers of combinations of stressors, it is possible for the
415 cumulative effects to saturate (A) because many stressors use same mechanism, or accelerate
416 if synergistic interactions combine and become increasingly synergistic with additional
417 stressors (B). In the case of saturation the number of tractable multi-stressor experiments
418 could be reduced. In the negative scenario, increasing number of stressors result in
419 acceleration of stress responses with high diversity of mechanisms and accumulation of
420 synergism making the number of required experiments intractable.

421

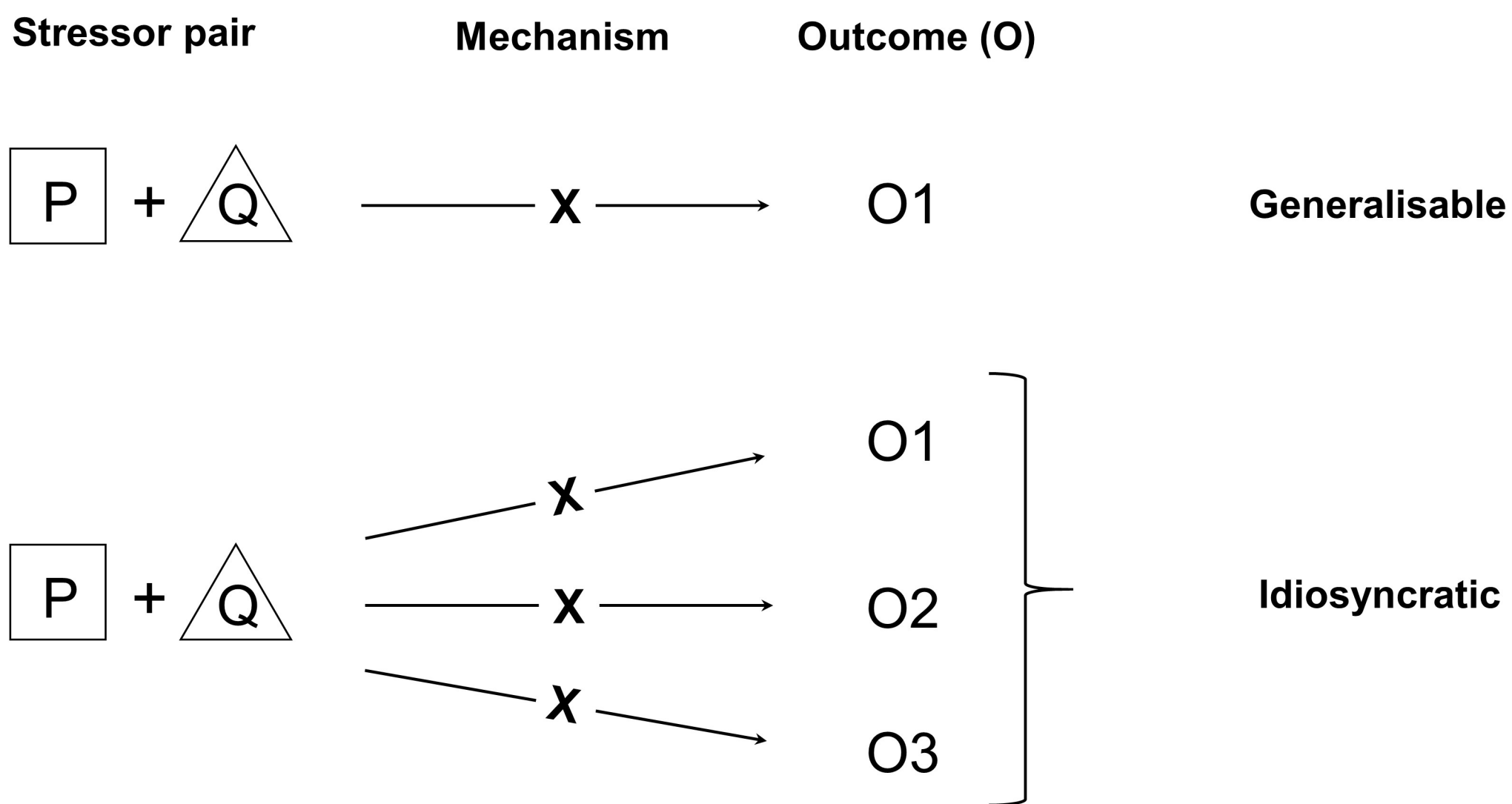
Figure 1



A)



B)



C)

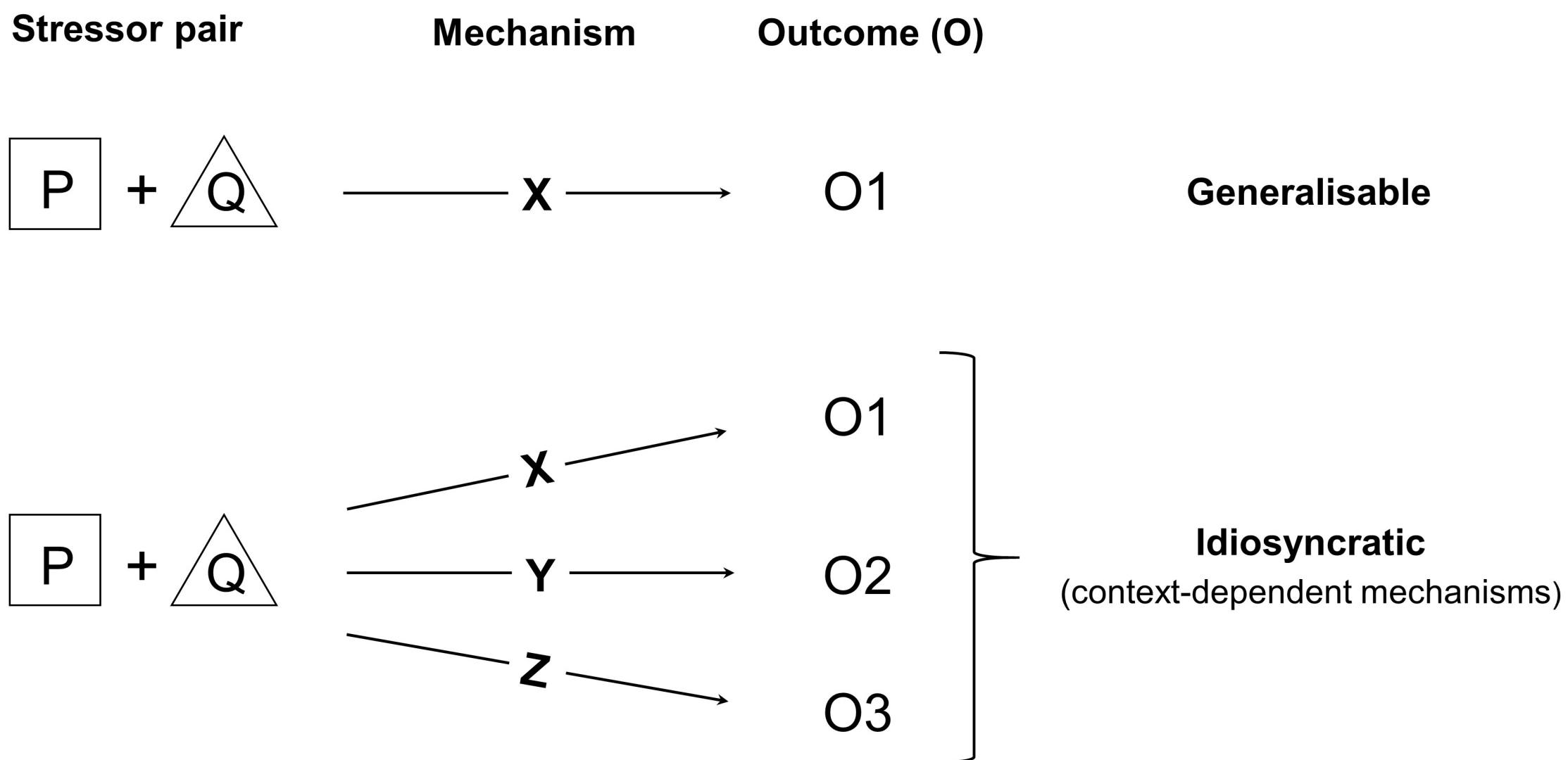
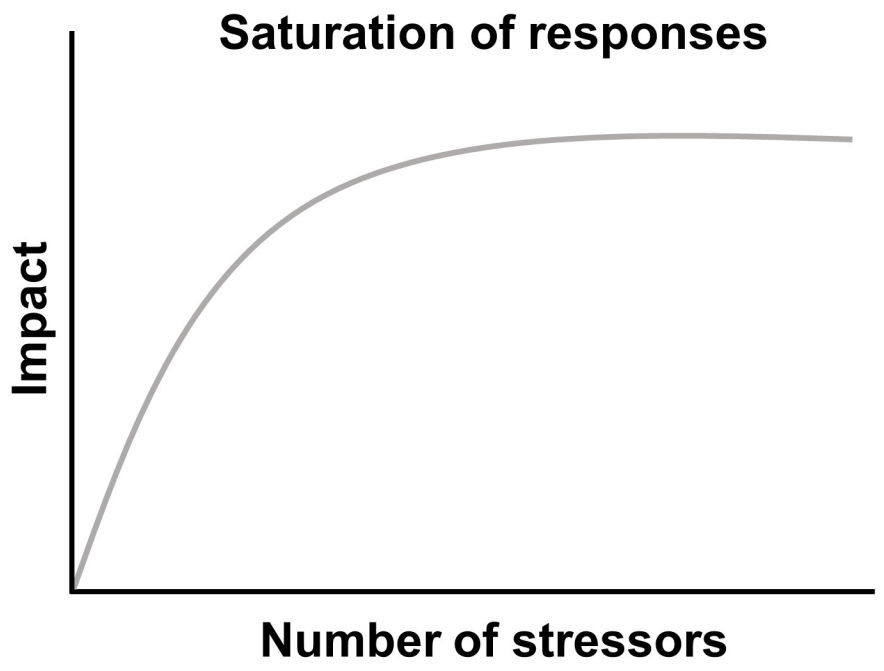


Figure 3

A)



B)

