A re-examination of variation associated with environmentally stressed organisms

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Variation is an essential feature of biological systems. Populations adapt to dynamic environments, in part, because of this variation. In this review, we re-examine phenotypic variation, especially in organisms living in polluted environments. A recent goal of ecotoxicology is to understand the sublethal effects of exposure to pollutants, e.g. responses to endocrine-disrupting contaminants. While variation is an inherent quality of organisms, variance is a statistical measure of the variation of a trait. Increased variance has been associated with organisms living at the perimeter of a population’s range, introduced into novel environments, or exposed to pollution. Some researchers have proposed increased phenotypic variance in exposed populations as an evolutionary mechanism, and others have suggested its use as a biomarker. While we agree that variance often increases in the exposed population, we also recognize that the opposite phenomenon occurs. That is, variance can decrease from exposure to pollution. Altered variance in the exposed population—leading to heteroscedasticity—could result in erroneous conclusions (Type II errors). We suggest that exposure to endocrine-disrupting contaminants could influence the health of populations in ways that are not always represented by measures of central tendency, and that variance and distribution should also be examined in environmentally stressed wildlife.

Key words: endocrine disruption/environmental stress/variance

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Introduction
The detection of alterations in physiological function or morphometrics following exposure to environmental contaminants is a fundamental part of the science of toxicology. Over the last few decades, biomarkers—which are phenotypic features that signal a change in an individual organism following contaminant exposure—have become important indicators of sublethal exposure. Recently, the interest in endocrine-disrupting contaminants has further focused the need for understanding individual and population responses to sublethal, chronic, low-dose exposure (Guillette and Crain, 2000). Alteration in endocrine functioning—especially during embryonic development—has the potential to alter permanently the morphology and physiology of a variety of systems (Bern, 1992; Guillette et al., 1995a; Gray et al., 1996). These changes can be as dramatic as altered sexual development (Jobling et al., 1998; Willingham and Crews, 1999), the formation of polyovular follicles in the ovary (Iguchi et al., 1990; Guillette et al., 1994), or alterations in phallus size (Guillette et al., 1996). They can be as subtle as changes in the type and number of cellular receptors (Bern, 1992) or enzyme function (Crain et al., 1997). Typically, changes in a response (phenotypic trait) of animals in an exposed population are compared to a reference population and analysed according to the differences in the central tendency of that response—the mean—and the variance around the mean.

If the alteration in endocrine signalling shuts down a system or dampens a response, such as a reduction in phallus size or depression in testicular androgen synthesis, the mean will decrease and is usually accompanied by a decrease in variance (Guillette et al., 1996). Likewise, exposures that reduce genetic variation in a population due to mortality or altered reproductive potential of individuals in a population will constrain phenotypic variation (Ricklefs, 1990; Russell, 1994).
**Variation and variance**

Variation and variance represent similar phenomena with related meanings. Variation is an inherent quality of organisms and a natural parameter of populations (Ricklefs, 1990; Russell, 1994). For each phenotypic trait, there is a spectrum of small alterations or differences such as changes in basic morphological patterns, protein shapes or enzyme actions. This variation is essential in biological systems as it provides the basis for adaptation and survival in dynamic environments. In contrast to variation, variance is a statistic that is a measure of biological variation. Phenotypic variance (\(s^2_{\text{phenotypic}}\)) is composed of genetic variance plus environmental variance (Ricklefs, 1990).

\[
\text{Variance} = s^2 = \sum \frac{(x_{\text{sample value}} - x_{\text{sample mean}})^2}{n_{\text{sample size}} - 1}
\]

**Variance in environmentally stressed populations**

Variance depends on multiple factors (both singly and in combination), including the magnitude and timing of exposure, the species and the type of contaminant. In environmentally stressed populations, variance is predicted to be greater in organisms exposed to heterogeneous environments in the wild. Variance has also been shown to increase where organisms are experimentally subjected to novel environments, such as exposure to contaminants (Forbes and Depledge, 1996).

**Increased variance in heterogeneous environments**

Organisms occupy a particular range or have a certain distribution because they are well suited to the physical, chemical and biological parameters of that area of the earth (Ricklefs, 1990). Speciation can occur at the perimeter of a population range, where the characteristics that comprise that range are more heterogeneous compared with the more homogeneous, central part of the range. Likewise, organisms occupying these range perimeters can exhibit greater genetic variance because they are struggling to adapt to the environmental stress of these heterogeneous areas (Myers, 1997).

Similarly, increased phenotypic variance in organisms has been associated with polluted environments, which are often heterogeneous with regard to the concentration or composition of the contaminant and the demographics of the population (Maurer and Holt, 1996). For example, the pulsatile release of effluent and the dilution effect of tributaries and variable rainfall could transform a river receiving paper mill effluent into a heterogeneous environment. Furthermore, changing weather patterns can alter the global or local transportation and deposition of pollutants for both aquatic and terrestrial environments.

The distribution and dispersal patterns of a population can increase the real-time heterogeneity of the environment for individuals of that population (Maurer and Holt, 1996). For example, organisms moving into and out of the highly contaminated regions of a landscape effectively increase the heterogeneity of the environment they experience. Similarly, non-mobile organisms living in relatively clean environments can be affected by the dispersal patterns and contaminant loads of their prey (Maurer and Holt, 1996).

**Increased variance in novel environments**

Organisms exposed experimentally to new environments exhibit increased variance in some measured traits (Parsons, 1989). For example, rice weevils, *Sitophilus oryzae*, bred on a non-toxic food type showed similar mean but increased variance in the levels of detoxification enzyme activity (esterase and glutathione-S-transferase systems) when transferred to novel, toxic and non-toxic foods (Holloway et al., 1997). The increased variance was greater following transfer to the novel non-toxic food, which suggests that just exposure to a new environment can effect an increased variance for certain endpoints.

Mandibular development in the common shrew, *Sorex cinereus*, was altered in an experimentally manipulated field population. Developmental stability was compared in shrews from the experimental plots (which had been logged) to the shrews from the reference plots. Eight measurements of the mandible in males and females were taken. Variation increased especially in the mandibles of the males from the logged site (Badyaev et al., 2000).

**Increased variance in contaminant-exposed populations**

There is a growing body of literature indicating a possible relationship between increased variance found in a measured trait and exposure to contaminants in populations living in polluted environments (for examples, see Table I). Most of these studies focus on the association between phenotypic variance and evolution (Lavie and Nevo, 1986; Koop et al., 1992; Bezel’...
Variation and environmentally stressed organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Contaminant</th>
<th>Phenotypic trait</th>
<th>Reference</th>
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<td><strong>Invertebrates</strong></td>
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<tr>
<td>House mosquito</td>
<td>Organophosphorus insecticide</td>
<td>Enzyme (esterase) activity</td>
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<tr>
<td>Rice weevil</td>
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<td>Fathead minnow</td>
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<td>Length, interocular distance, head width</td>
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<td></td>
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<td>Anal fin length and segment number</td>
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<td>Florida gar</td>
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<td>Common shrew</td>
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<td>Humans</td>
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Contaminant-exposure that results in decreased reproductive potential, altered development or creation of essentially clonal populations (severely reduced genetic variance) are considered subtle effects of contaminant-exposure when compared to sterility, teratogenesis or species extinction. Increased phenotypic variance in the exposed population could be the first steps in the evolution of a population to a changing environment. However, if organisms cannot adapt and thereby survive to reproduce, the population may not evolve but become locally extinct.

**Variance as a biomarker**

Several studies have suggested that, following exposure to contaminants, variance can be a more sensitive biomarker than the mean activity levels of various detoxification enzyme systems such as esterases or glutathione-S-transferases (as in the rice weevils described earlier; Holloway et al., 1997). The esterase activity of a wild population of mosquito, *Culex pipiens*, was examined following exposure to an organophosphate insecticide and compared with enzyme activity levels in a control laboratory population (Callaghan et al., 1998). Increased variance and mean esterase activity was measured in the field population that was known to be resistant to the insecticide. Interestingly, this increased variance persisted following long-term genetic selection, resulting in decreased levels of polymorphism in the exposed population (Callaghan et al., 1998).

**Variance and data analysis**

Should we always conclude that two populations are not different because they lack a difference in the mean value for the measured trait? For example, we measured the anal fin length of a group of female mosquito fish from two rivers, one polluted and the other a reference site. Previous studies had reported that many females in
the polluted river exhibited elongated anal fins (masculinization), presumably due to the exposure to an as yet unidentified androgenic pollutant(s) in the paper mill effluent-tainted water. Our recent examination of female fish from this polluted river found no mean difference in anal fin length. However, there was a difference between sites in the distributions of this trait, with the exposed population having an increased variance about the mean. If 15% of the exposed population had masculinized anal fins, would it be biologically accurate to conclude that the population exposed to paper mill effluent was not affected? Examples like this and those in the following sections suggest that we should analyse and interpret our data using both variance and distribution, as well as measures of central tendency.

Not all responses to environmental stressors or measured traits follow the pattern of increased variance in the exposed population. There were true differences in the mean without an associated change in distribution in many of the endpoints measured in the following research studies. Although increased variance in organisms living in contaminated environments does not occur all the time, it seems common enough that we should consider distribution as an important population endpoint. Otherwise, we could mistakenly commit Type II errors; that is, accepting the null hypothesis when it is actually false.

**Exposure to pesticides:** The American alligator, *Alligator mississippiensis*, living in Lake Apopka, Florida has exhibited altered plasma concentrations of steroid hormones, decreased phallus length in the male alligators, and developmental abnormalities in the gonads of both sexes (Guillette et al., 1994, 1995b, 1996). These effects have been associated, experimentally, utilizing treatment studies with environmental pesticides such as toxaphene, dieldrin, Dicofol and dichlorodiphenyltrichloroethane (DDT; and its metabolites p,p’-DDE and DDD) that are pervasive in the food chain of Lake Apopka organisms and in the eggs and serum of the alligator (Heinz et al., 1991; Guillette et al., 1999).

To investigate if the reproductive abnormalities in the alligators were found in other organisms living in this lake, we examined the Florida gar, *Lepisosteus platyrhincus*, collected from Lake Apopka, and a reference site, Lake Woodruff National Wildlife Refuge. Although there was no difference in mean values for standard length, body mass and liver mass of female gar, we observed an increase in the variance for each of these parameters in Lake Apopka fish (Figure 1).

**Exposure to cattle feedlot effluent:** Cattle are often given natural and synthetic anabolic steroid hormones to control growth rates, fat to muscle ratio, and carcass quality (Apple et al., 1991; Mader, 1998). Although these hormones are administered in high concentrations, relatively low concentrations remain in the meat that humans consume (Lone, 1997). We postulated that these hormones are excreted, enter surrounding watersheds, and alter the reproductive biology of organisms living there. A similar phenomenon has been reported for natural and pharmaceutical oestrogens released in sewage treatment plant effluent in Great Britain (Harries et al., 1996; Jobling et al., 1998). To investigate the possible effects of excreted anabolic steroid hormones on aquatic organisms, wild, endemic fathead minnows, *Pimephales promelas*, were captured downstream from cattle feedlots in Nebraska and compared with fish taken from a reference stream. No mean difference was found in body length, whereas interocular distance and head width were decreased, and greater variance was observed in all three parameters of male fathead minnows from the contaminated site compared to the reference site (Figure 2).

**Exposure to paper mill effluent:** Masculinization of secondary sex characteristics of female mosquitofish, *Gambusia holbrooki*, has been associated with exposure to paper mill effluent (Howell et al., 1980; Davis and Bortone, 1992). These studies have indicated that exposed females exhibited elongated anal fins similar to male mosquitofish. Males have highly differentiated anal fins, with anal fin rays 3–5 elongated to form an intromittent device (gonopodium) for the transfer of spermatozoa into the female’s reproductive tract. Each anal fin ray has crosswise-oriented segments. The number of segments is a sexually dimorphic characteristic, with males having a greater number than females. Differentiation of the gonopodium is androgen-dependent and does not normally develop in the female (Rodriguez-Sierra and Rosa-Molinar, 1990; Rosa-Molinar et al., 1996). Recently, we have examined this phenomenon in the Fenholloway and Econfina Rivers in Florida. These rivers share a common headwater, and travel similar distances to their termini in the Gulf of Mexico. However, water quality in these rivers differs greatly due to the presence of a paper mill plant that releases approximately 50 million gallons per day into the Fenholloway River. During the dry season this effluent can constitute up to 80% of the river volume (Bortone and Cody, 1999). Sexually mature female mosquitofish exhibited no difference in anal fin length, but the Fenholloway females had a greater number of segments per longest anal fin ray compared with Econfina females (Figure 3). Moreover, increased variance was observed in both elongation and number of segments in the anal fin (Figure 3).

**Human populations:** The phenomenon of increased variance in the contaminant-exposed population extends beyond wildlife to the human realm. Pre-school children from two populations of agricultural-based communities in Mexico were examined for
possible developmental effects from pesticide exposure (E. Guillette et al., 1998). One population lives in the mountains, and farms using traditional methods that do not utilize pesticides, whereas the valley population has embraced modern agricultural materials and methods. Dramatic differences were noted in a number of behaviours when the children of these regions were compared (E. Guillette et al., 1998). However, there was no difference in the body mass, standing or sitting heights between populations. Also, there was no mean difference in the head, chest and upper-arm circumferences, suggesting no major differences in growth. However, there was greater variance observed in these traits in the valley (exposed) population when compared with the mountain (reference) population (Figure 4). Interestingly, the increased variance in such parameters as head, chest and upper-arm circumference could help explain the behavioural differences noted in these children, as many of the alterations were related to hand–eye coordination and stamina.

Conclusions

The aforementioned studies, ranging from invertebrates to humans (see Table I) suggest that we need to re-examine how our data are analysed and interpreted. For the examples taken from the gar (all parameters), human (all parameters), mosquito-fish (anal fin length/standard length) and fathead minnow (length) studies, the mean values were not different, yet the variance about the mean was greater in the measured trait of the exposed population (Figure 5). In contrast, with both the fathead minnow and mosquito-fish, the mean values for some of the parameters were different between the exposed and reference populations. In the mosquito-fish, the mean value (segment number) increased in the exposed population, with the variance increasing disproportionately. The mean value increased by approximately 68%, whereas variance was greater by 469% in the exposed population (Figure 5). Although the mean (here summarized as the average of the means for interocular distance and head width) decreased in the exposed population of fathead minnows by ~14%, the variance actually increased by 1037% (Figure 5).

The observation that variance is increased in the exposed population may at first seem counterintuitive. If greater variance is expected in a healthy population, and a population’s stability is, in part, a function of variance (and the size of a population), how do we explain increased variance in the exposed population, and why might this be a concern?

In a healthy population, one would expect an optimal distribution in any measured trait (Figure 6). The observed
variance is phenotypic variance, which is composed of genetic and environmental variances. As the basis for this optimal distribution, we would expect a greater genetic variance to have evolved in our reference (stable) populations, and we postulate that is the case.

What may be forgotten is that phenotypic variance has an environmental component. That is, the measured phenotypic variance has a genetic component that is acted upon by the environment (Ricklefs, 1990). Populations that have achieved a theoretically optimal distribution for a trait, therefore, would be expected to exhibit lower phenotypic variance because they are adapted to their surroundings and would perceive it to be a relatively more homogeneous environment.

Organisms living at the perimeter, and hence more heterogeneous part of the population range, are expected to have increased genetic variance, when compared with organisms living in the more central, therefore homogeneous part of the range (Ricklefs, 1990; Myers, 1997). Increased genetic variance evolves as a population struggles to adapt to this heterogeneous environment.

Organisms living with pollution can occupy heterogeneous environments. In particular, polluted environments with point-source origins are heterogeneous due to the pulsatile release of the effluent, variability in the dilution rates, and the changing composition of the effluent. Compounding that heterogeneity is the fact that organisms are mobile and tend to move in and out of the more polluted areas of the range or variability in weather patterns can affect transport, distribution and deposition of contaminants from the atmosphere (Maurer and Holt, 1996).

A reference population with its evolved optimal distribution is at equilibrium or steady state (Figure 6). When a population is stressed, the variance increases and the frequency decreases as individuals attempt to adapt to the altered environment. Environmentally stressed populations could eventually develop one of the altered distributions shown or become extinct.

Increased phenotypic variance represents an increase in energy use in the population that goes to physiological factors other than reproduction. That is, energy budgets are limited in many populations requiring the partitioning of energy in individuals between somatic maintenance, growth and reproduction (Roff, 1992; Stearns, 1992). If organisms in a population are required to increase energy utilization in such a form as increased phenotypic expression of a given trait(s) (e.g. additional enzyme activity or modified growth), then this will have a direct cost to the population in the short term, and probably also in the long term. Over time, the distribution and/or mean could be altered, resulting in a new, yet less stable, steady state.

In conclusion, increased phenotypic variance may be an important early sign of perturbation in a population. Thus, we might use the variance statistic as an early indicator of exposure to subtle toxicants, such as endocrine disruptors. Statistics based on measures of central tendency remain critical to the analysis of data. Yet this metric cannot, as we have seen, be the only criterion by which we compare populations. We know that not all members of a species are the same; variation is a characteristic of all
organisms. While measures of central tendency are important statistics, we risk ‘seeing’ a population as a single value, instead of the continuum of values for a phenotypic characteristic. A population’s resilience is correlated with its phenotypic variance (Petterson and Allen, 1998). While an increase in variance is not observed for every trait measured or type of pollution that organisms are exposed to, data we have presented here from our own and other’s research suggest that distribution and variance should be analysed. For the relatively subtle effects of exposure to endocrine-disrupting contaminants, perhaps examining the distribution and variance of a response could yield more useful information about the health of a population or ecosystem.

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