

Further experimental investigations on variation of types, and on the nature of quantitative differences in Daphnia types.

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Abstract

The following is a translation from the German of Richard Woltereck's classic paper¹ introducing the idea of reaction norms; a concept which encapsulated the now-ubiquitous idea of a genotype-by-environment interaction, and contributed to the idea of the developmental program.

Woltereck begins with a brief discussion of Lamarckist and Selectionist views on heritability, the influence of the environment (he uses the term "Milieu"), and variation of types. This is followed by a discussion of methods for understanding these concepts in biological systems. A research plan is outlined for distinguishing between these possibilities in a fashion to be generally convincing among the diverse factions. Data following the initial steps of this research plan are presented. An introduction to the model system of Daphnia is provided, and in particular the interesting response of head morphology to seasonal variation ("cyclomorphosis").

The basic observation of consistent responses to the environment among clonal Daphnia is presented and analyzed in light of both temperature and nutritional variation, and in terms of physiology and morphogenesis. Woltereck goes on to determine that manipulating food quantity is sufficient to recapitulate this variation in the laboratory. He calls the response of a genotype to environmental variation a reaction norm.

Woltereck develops his theme by comparing different genotypes (captured under Johannsen's terms of "Biotype" and 'elementary type') in their reactions to different environmental conditions. He further contrasts the observed data to the expectations under Johannsen's simple expectations of the independence (meaning here additivity) of contributions of genotype and environment to mean values of phenotypes. He finds that this expectation of independence is not borne out in the data. Moreover, the differences between environments varies from negligible to dramatic even for the same compared genotypes, and thus the differences do not comport well with a discontinuous model of mutational variation. Instead, the differences between types are continuous and

difficult to predict from bulk genotypic or environmental differences.

Woltereck then shows that similar dynamics underlie variation of sexuality frequency differences in Daphnia genotypes, indicating that his results are not a simply a peculiarity of the head-height character.

The paper finishes with some experiments by which Woltereck aims to evaluate hypotheses of generating heritable quantitative variation (in the "genotype") through environmental exposures. In these cases it appears that he has anticipated and clearly described what was later called "genetic assimilation of acquired characters" by Waddington. Woltereck shows this for the characters of presence-absence of nebenaugen, for crown toothlets, and briefly for both head height and sexuality. Some of these characters at least are atavistic.

[TO BE FLESHED OUT]

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Outline

Preface: General. — Necessary Materials. — Isolation of the Elementary Types. — Plan of Investigation. — Observations on the Crossing and Selection of Daphnia.

1. Ascertainment and Analysis of the Quantitative character "head-height".
 - (a) The basis of different head-heights.
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 - (c) Genotypic basis of head-height and different genotypes.
 - i. Genotype = Reaction norm
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2. Analysis of the phenotypic and genotypic differences of the Quantitative character "sexuality"
 - (a) Exact determination and quantitative measurement; causes of the differences
 - (b) The beginnings of genotypical distinction — Natural Transitions.
 - (c) Artificial transitions
 - (d) Differences of reaction of generations

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¹The text is drawn from pages 110-173 of the *Verhandlungen der Deutschen Zoologischen Gesellschaft*, vol. 19, 1909, as digitized by the Biodiversity Heritage Library (<http://www.biodiversitylibrary.org/item/182001>). I copyrighted the translation under my own name for lack of a better idea.

- i. Specific generation sequences
- ii. Their relevance to natural environments
- 3. Undertakings and experiments concerning regressive variation
- 4. Experiments towards generating new genotypical quantitative characters through the influence of the environment

Appendices:

1. The causes of continuous variation in Johannsen's experiments and my own
2. On the metabolic physiology of Cladocerans
3. Examples of partial alterations of the reaction norm
4. Sexuality and generational cycles

Preface

From the results of many experiments, in particular botanical studies of inheritance of the last century—on the one hand the Mendel's crosses, on the other the inbreeding experiments of Johannsen, and most lately the famous mutations of de Vries—we have derived a new concept in the existence of the variation of types. This concept, which is championed especially by de Vries and W. Johannsen², stands in nearly-irreconcilable contrast to the Lamarckist inclinations of today's Botany, and also of most zoologists, regarding whether they consider themselves Lamarckists or Selectionists.

We hear the following: the elementary types (not concerning ourselves with the artificial umbrella term of systematic “species”) differ from each other through the assortment of predispositions, determinants, or genes, which are inherited in their germplasm. So far the “exact doctrine of heredity” (Johannsen) corresponds to the older teachings, especially Weismann. Moreover, this doctrine provides the most brilliant evidence for the existence and continuity of the germplasm-elements that Weismann could wish for, with regard to the correctness of his theoretical ideas. The dissent of the new teachings concerns only the variation inherent in these elements:

1. These hereditary elements are of fixed quantities, which do not show the smallest variability in siblings (respective in germplasm to their parents), or show any gradual variation across many generations. A change in the elements, and thereby the heritable characters of the elementary type, follows exclusively through spontaneous mutations; fluctuating, individual, or continuous variability is negligible with respect to the heritable nature of types.³

²Compare especially Johannsen's new book: *Elemente der exakten Erblichkeitslehre*. Jena, G. Fischer, 1909 (515 p.).

See also the report of Arnold Lang in this journal. Finally I would like to draw attention to the important work of the Heidelberg botanist Georg Klebs, without which I could not have completed the present work. Klebs has demonstrated the exceptional scope of continuous, environment-dependent variability in several plants (*Sempervivum*, *Sedum*). See also his “Studien ueber Variation” (*Archiv f. Entwicklungsmech.* 1907)

³translator's note: these terms appear to be taken directly from De Vries' writings in *The Mutation Theory*.

2. The nature of change of the hereditary elements, and therefore the direction of these mutations, does not have a causal relationship to the local conditions, or environment. Extreme changes of environment may prefer the emergence of certain mutations, and thereby “disturb the equilibrium of the germplasm”, but the thus-unleashed variation of germplasm and type does not indicate any causal relationship of the mutation to the environment.

The environment in no way affirms the direct inheritance of variation of type (Lamarckism), and even its indirect action (by harvesting the most adequate variants; that is, Selectionism) is secondary. Selection does not decree the multitudes of always-available, always-fluctuating germ-variants (and absolutely cannot impart a direction on the organs, tissues, or germ cells); its scope is restricted to the occasionally-appearing final mutations. Here it will eradicate the unworkable mutants through the preference for certain forms, at least so far as they have not already left the stage through inviability or sterility.

Therefore: there is no continuously-arising variation and equally no continuously-arising evolution.

And: there is no determining influence of the environment, except through the excision of unfavorable mutants.

These are hard blows against that conception of the development of the organic world in which we are tutored. However, the zoologists present so many illuminating examples of finely-graduated arrays of forms and adaptations that it is difficult to accept a conception of discontinuous development, and that by these little steps of type-variation the influence of the environment (whether direct or indirect) can be explained away, without giving up our lone mental anchor to an unmetaphysical understanding of organic development.

However, the advocates of the “exact doctrine of heredity” support themselves with analytical experiments, and therefore they see all the rich body of facts that have accumulated since Darwin as inconsequential—dashing our hopes, like a tanner who loses his hides [translator's note: ???]. Of course: experimental analysis is that rich goal towards which observational facts are only the smallest steps.

Therefore, if we want to meaningfully fight against the new doctrines we must use the weapons of the enemy, above all analytical experiments. Then we will compare the worth the “old” conception: continuous variation of type under a determining environment; to the “new”: discontinuous variation without a determining environment. Then it will be clear that the latter conception, of mutations, is proven experimentally.

Necessary Materials for Investigating the Variation of Types.

If we wish to assess precisely whether organic properties vary continuously or in jumps, it would be best to make use of predefined quantitative characters (e.g. length measurements). In these characters, variation can be numerically and unambiguously specified. It is my opinion a weakness in the new doctrine that Johannsen presents, that he considers only qualitative categorical characters (e.g. color) that are altogether subjective. We must take greater pains to eliminate

the influence of crossing and sex in our experiments, such that only sexless, parthenogenetic, or self-fertilizing organisms are appropriate. In this sense, it is indispensable that we study variation and heredity in “clean lines” (Johannsen).

Third, finally, it is preferable that we only have to do with organisms that are very diverse in form [trans.: alternatively, numerous], in order that small (but heritable) differences can be discerned. The comparative study will then concern itself with forms that show no substantial difference, other than one or a few quantifiable contrasts, which in comparison constitute elementary types (“Biotypes” for Johannsen).

The familiar Danish botanist succeeded in cleanly identifying such elementary types in great numbers out of landraces of grains and beans. We require similarly numerous and diverse animals for zoological studies to design a comparable experiment, for instance Daphnia or another Cladocerans that are available in our lakes and ponds. For an account of landraces in Daphnia, I can recommend my own work showing different heritable Biotypes⁴.

Both of the other requirements are fulfilled either in Johannsen’s work, or in my Daphnia. Johannsen isolated “clean lines” through self-fertilization, whereas I used parthenogenetic propagation under controlled, constant culture conditions while removing the eggs with ephippia (egg casings generated during sexual reproduction), such that the fertile eggs develop into ♀♀. Thus the correctly-timed isolation and robust feeding of these ♀♀ suffices across many Daphnia races to maintain clean lines (in some cases for this purpose, the sibling ♀♀ without ephippia should be excluded). Almost invariably under favorable conditions, the animals revert from laying more ephippia to making parthenogenetic eggs (subitan eggs), and indeed almost without exception they are ♀♀ individuals that arise out of such (“post-ephippial”) virgin eggs. The appearance of ♂♂ in unattended cultures can be dismissed, so long as no ephippia appear; to this end, new generations were obtained by descent through single ♀♀ individuals, to entirely exclude this possibility.

As is well known, Johannsen used as quantitative features the length of beans and the serration of barley varieties. In Daphnia, the length of the carapace spines, the height of the head, the facial angle, the spine angle (compare figures 1 and 2), and sexuality (see below) can serve as quantitative characters.

Thus are the materials which Johannsen used and my own Daphnia populations sufficiently comparable, at least in these three critical points, in spite of the totally unrelated morphologies of higher plants and metazoans.

Isolation of the Elementary Types

As is well known, Johannsen proceeded from the premise that he isolated variants comprising different elementary types or Biotypes that were mixed in an initial, apparently uniform population. These types proved themselves through the heritability of coherent, unchanging features. The progeny of each individual, for instance the largest and

smallest, show similar means and the same range of variation as their parents. (Johannsen’s thrilling results were, of course, that selection on the variants of an elementary type does not reveal any influence of heritability on variation, specifically if the same conditions are used year-to-year. This result shook the foundations of research of statistical variation and heredity, as practiced by the Galton-Pearson school. Specifically, the meaning of the famous regression laws, which to many were the basis of all exact heredity and speciation research, was called into question by Johannsen’s findings.)

With Daphnia, obtaining elementary types is easier on one hand, and one the other more complicated. Easier, because most studied natural populations (inhabiting ponds and lakes), are found to be consistent and homogeneous. In the same body of water, in a more than square kilometer pond by Torgau, I could find two Biotypes in an apparently homogeneous population of *Daphnia longispina-galeota*. These types showed not-insignificant differences in their “relative phenotypes” and inheritance. Many bodies of water have yielded similar results, so I assume that it is the same for the Danish populations of Daphnia studied so exhaustively by Wesenburg-Lund.

Thus it is not surprising that in a single lake multiple elementary types of *Daphnia longispina* and *cucullata* coexist, but rather that only one apparent biotype of each elementary type or subtype exists. And therefore we must confront the complicated side of our question (compared with the bean and barley biotypes maintained through selfing), concerning the poorly-illuminated basis of the identity of types.

In considering Daphnia types, in which sexual reproduction and diapause (dauer stage) play a greater or lesser role, we would expect that the mixing of local forms is substantial. In reality, we observe essentially no type mixing. In the case of such mixing, we should always encounter inconstant forms of variable descent, rather than the striking constancy which the forms show from year to year.⁵

The mixing of local types with strange biotypes appears much less important than the mixing maintained within some local types at the end of the breeding season. Through this general admixture within individual germplasms, the differences within the local types will be balanced out as generations proceed.

The transition between obligatory sex and total parthenogenesis takes at least a year. With most Daphnia the lakes show a greater or lesser proportion of the ♀♀ are maintained as parthenogenetic, while the other individuals (majority or minority) go over to the production of dauer or diapause eggs and ♂♂.

It would be worthwhile also to make a comparative study of the means of inheritance in varieties with different degrees of sexuality. In particular, it would be good to bring up those populations which have given up sexuality entirely (for instance, *Bosmina* and *Daphnia longispina-hyalina* of the great alp lakes). In the case of these last forms we must expect the population to be split up into more biotypes.

For the task at hand, these considerations are of theoret-

⁴Verh. d. D. Zool. Ges. 1908, vol. 18, pp. 234-240

⁵see my paper Verhandl. 1908, p. 239.

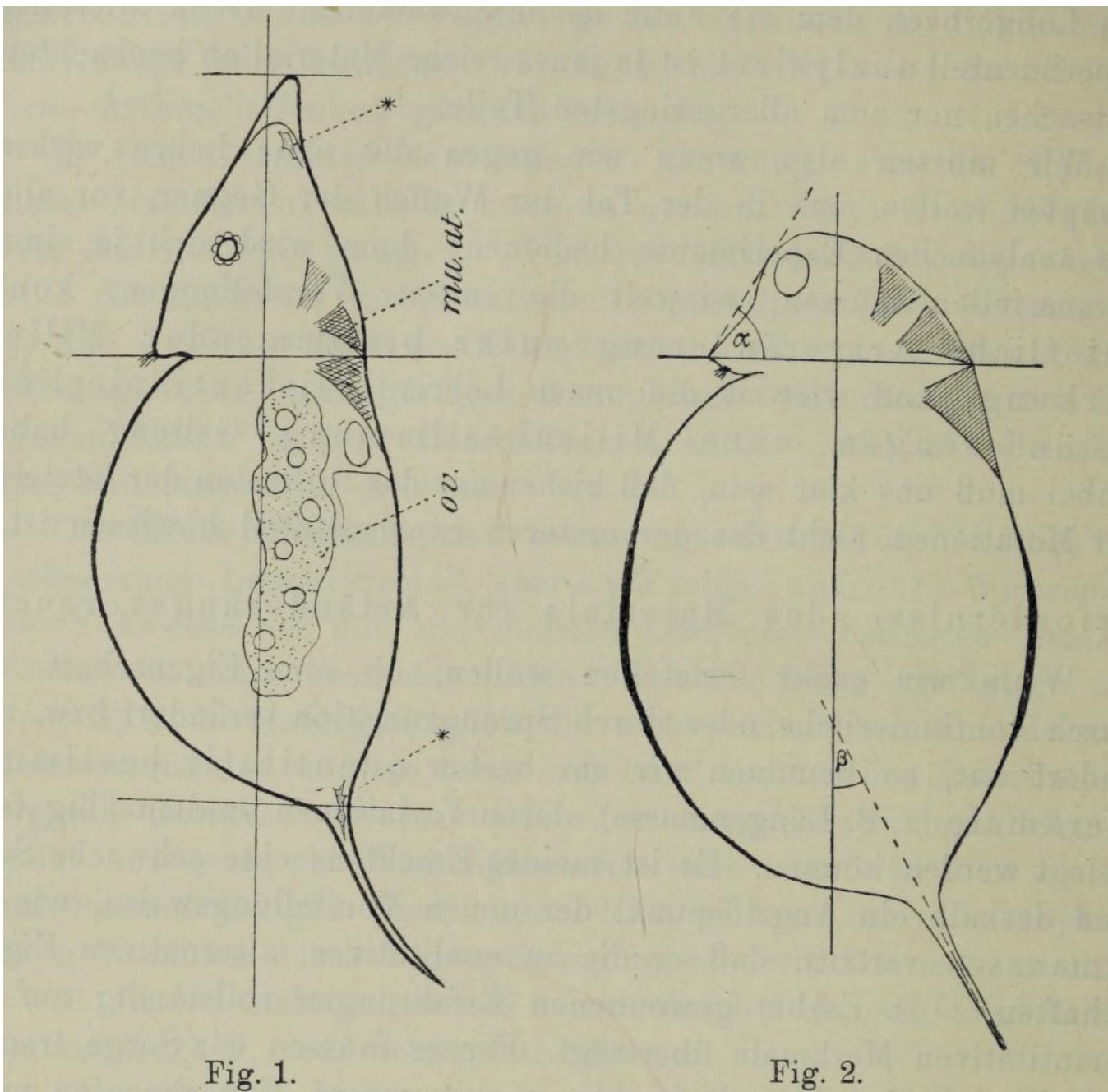


Fig. 1.

Fig. 2.

Fig. 1. Side view of *Hyalodaphnia cucullata*-♀ of intermediate height. The superimposed axes allow the determination of head height. One obtains a single useful "head-base" by drawing a line connecting the point of the rostrum (bottom edge) to the border between the second and third antenna-muscles (indicated *mu. at.*). The midpoint of this line yields a perpendicular line describing the body's long axis, along which we can measure head height and the length of the carapace as shown. Out of these individual measurements one can compute the "relative head height", expressed as a percentage of carapace length. It is necessary each time to draw from a photograph, in order to quickly and accurately capture the outline (including the head-base) on the slide, while at the same time preserving the life out of water of the animal in question. Fig. 2. *Daphnia longispina* from the Lunzer Untersee [trans.: an alpine lake in Austria]. Angles of the axes indicate the facial angle (α) and the spine angle (β).

ical interest (in the selection and orientation with respect to the working material), not necessarily practical meaning. In each of my cultures I deal with single biotypes, because if I have selected a lone ♀, the following generations will necessarily be parthenogenetic. These generations represent "clean lines", like those that Johannsen studied.

They are thus the first instance of working material for the experimental analysis of the quantitative character, whose change (whether continuous or discontinuous, and whether with or without determination from the environment) we should study.

Research Plan

The success of such an undertaking requires a systematic approach. It must be carried out in such a way that the local forms compared represent elementary types and not only slight variations of the same type, requiring the following tasks:

1. Analysis of the collected modifications that the various characters of a given biotype can show, which are thus caused by the conditions of the environment. We will thereby understand the various potential forms (the "Phenotypes" of Johannsen) of each character on the one hand, and on the other define the heritable "genotypic" differences between biotypes, in order that we may compare these biotypes under like environments. To eliminate the various influences on individual cases of embryonic development, we must not compare individual measurements, but rather the averages of a rather large count of age-matched individuals of the same generation and environment.

With that said, this first task of analyzing available biotypes depends upon our ability to focus on a single character or trait (for instance, head-height), in three sub-tasks of increasing difficulty:

- (a) Exact determination of the range of variation of the relevant character under all possible conditions of the environment.
 - (b) Exact determination of those conditions of the environment, which elicit individual modifications of the character in question.
 - (c) Exact determination of the (heritable) differences, which are shown in the collected modifications of the relevant character between different biotypes under the same conditions.
2. After these initial steps, a second area of work constitutes the testing of thus-determined genotypic differences of two elementary types through crossing. This is to answer the question of whether the ascertained quantitative variations in characters resolve into intermediate forms, or whether they follow (and according to which rules) the rules of dominance and segregation.
 3. Further, we must observe whether biotypes isolated from clean lines can give rise to extreme variants under extended selection.

4. Finally, we must test whether and how far the continuous action of specific conditions of the environment can achieve the change of a character within a biotype.

My experiments have been carried out at this point over the course of three years and concern themselves more or less with the set of tasks under the first heading above. I have also made a beginning of my attack on the remaining questions, but unlike the work presented here, they have not yet achieved clear and final results. An extensive description of my attempts will appear as soon as possible in book form, together with the data itself, laying out my underlying interpretations and conclusions.

Observations on crossing and selection in *Daphnia*.

Concerning points 2 and 3 (but little treated in the above enumeration) I will make a few observations here.

Concerning 2, the attempts at crossing have run into obstacles related to methodological difficulties in obtaining perfect data, specifically in generating a sufficiently large number of progeny to obtain average values of the quantitative traits among mongrels. And indeed, whether these progeny are equivalent, e.g. the same culture and generation giving rise to equal males and also perfectly equivalent females. We will therefore see in the following results that the determination of the genotypic values of the quantitative characters as well as the definition of the 'grades' of the environment, and also the count of the generation cannot be gone without.

The relatively few mongrels which I have obtained from numerous experimental crosses between *Daphnia longispina*- and *cucullata* local types show intermediate characters, but an unambiguous evaluation is at this point, as written before, impossible. I address these points here only to alert other researchers that the inviting mongrelization-experiments (at least for quantitative characters) will only yield value from an extensive phenotypic and genotypic analysis, and then only if the obtained material is adequate to the questions under study (average values, the equivalence of the parent stocks).

Concerning 3, the large majority of my experiments attempting to call forth extreme variants under selection upon clean lines have yielded negative results. This is very similar to the results of Johannsen. On the exceptions from this rule, and on the various experimental series where changes have occurred, I cannot at this point provide much clarity. Therefore, in the following pages I will only touch upon these points so far as I believe will serve future researchers.

I will briefly consider the observed mutations, through whose isolation (selection) one can obtain what are actually strongly changed heritable lines, in another context (Seite 137). They are certainly of immaterial meaningfulness in terms of the generation of types in *Daphnia*.

Now, the important question concerns whether a continuous character can be changed only through continuous selection on variants (those increasing or decreasing it, for instance). I wish to emphasize the necessity of clearly separating the influence of the selection from that of the envi-

ronment in changing this character. This proved quite difficult in *Daphnia*; it seems doubtful whether factors of the environment (including during embryonic development) can be entirely eliminated in the study of selection. Instead, the promising studies are those in which selection factors can be determined through, for example, comparing the effects of different environments on unselected populations to age-matched individuals who have been selected for the strongest deviation in the character. The influence of selection then appears out of the difference between the simple and the combined methods. [translator's note: ?] However, we have not yet come so far. We will see that one type-changing environment (of four) at this point has only the first beginnings of something to show, considering so far as the artificial evocation of new genotypes. On the selection of new variants, consult Seite 158.

However, as we will see next, from the analysis of the existing *Daphnia* genotypes we may already have arrived at compelling conclusions about the nature of the variation of types.

I. Exact determination and analysis of the collected phenotypes of the quantitative character "head-height".

We understand "phenotype", like Johannsen, to signify the average value of a character under some circumstance, for instance the middling length of a stalk in an evenly watered and manured field of rye. This phenotype can be singular, if all the plants belong to a single elementary type, or only apparently singular, when more heritable ("genotypic") different elements are planted, when we deal with a mixed population. However, in the following work we concern ourselves solely with phenotypes whose singularity can be guaranteed through previous hereditary experiments. To simplify the picture further, we prefer to focus on some particular character, namely the head-height of *Daphnia* land-races (which we treat as elementary types). Further, we obtain the 'relative' head-height by also considering the length of the carapace (compare Fig. 1 and relevant explanation).

Our first task is now to compute the phenotype of head-height by measuring age-matched samples of 100 examples (♀♀ or ♂♂) from natural populations, from which we obtain a frequency curve and can then compute the desired average (phenotype).

Such a calculation would only have worth if we are able to obtain age-matched animals (that have, for example, gone through a like number of molts), as they might also change their relative head-height over their lifetime. We must confine ourselves in this analysis to roughly similarly "grown-up" animals, preferably considering only the ♀♀ , for the purposes of constructing our curve. We may nonetheless in many cases obtain multimodal curves, if for example adult ♀♀ from different generations are available, that can vary in their head-height in the early year particularly.

It is therefore not permissible to compare the "natural" frequency curves with curves generated from cultivated materials, so much so that we will never make comparisons involving individuals that are not from the same generation

and age class.

If we perform a similar experiment in wild animals at different times of year, across many *Daphnia*, we reliably find quite different head-heights in spring, in summer, and in autumn/winter. Thereby, the frequency-curve and its mean continually move; we obtain a spring curve, a summer curve, *et cetera*. Or more precisely stated: the curve (and with it, the phenotype) of head-height shifts with time of year from Spring until September to the right, only to return once more to the left (Fig. 3).

Wesenberg-Lund has described the change of head-types across many *Daphnia* of the Danish lakes, giving us a characteristic example of cyclomorphosis. These samples of animals are of course taken directly from the lakes in question, not from cultures. These observations are thus very informative, collected as they are across many years, in descriptively characterizing the forms of local races. However, in seeing beneath the description to the particular heritable nature (the genotype) of an elementary type, we cannot do without cultures.

In our cultures, we can artificially regenerate all of the different head-heights that we observe in lakes over the course of a year. We must, of course, take care that our culture conditions⁶ be correct and appropriate. I have accomplished this in various attempts to obtain pure pelagic *Hyalodaphnia culicula*.

a. The causes of different head-heights.

There are three different principal factors from which the different head-heights in the space of a Biotype are derived (similar to other characteristics of form, such as spine length, spine angle, brow angle, and overall size): feeding, temperature,, and generation number.

External factors Until this point the role of temperature as a biological factor, at least since Wo. Oswald's⁷ treatment of the subject, was thought to be related to its inverse relationship with water friction (at higher temperatures friction is lower). Consequently, planktonic organisms require a higher buoyancy at higher temperatures to remain in suspension than they require at lower temperatures.

The buoyancy of *Daphnia* bodies is apparently increased by, among other things, the body processes (on one side the head-height dependent "helmet", and on the other side the mantle-spine); or also by reducing overall size, which increases relative surface area. The hypothesis that relevant body alterations may be triggered or caused by temperature changes is therefore obvious.

However, my cultures show to the contrary that temperature plays only a secondary role in these alterations. Further, head-height is not altered through changes in internal friction. In coming to this last conclusion, I rely primarily on results from the addition of quince mucilage to the medium, which is better borne by the animals than supplementation

⁶Consult my note on the rearing of *Daphnia* in the Intern. Rev. d. ges. Hydrobiol. 1908, Volume 61, 871-874.

⁷From his numerous writings on this subject, one is especially relevant: "Exper. Unters. ueber den Saisonpolymorphismus bei Daphniden" (Arch. f. Entwicklungsmech. 1904).

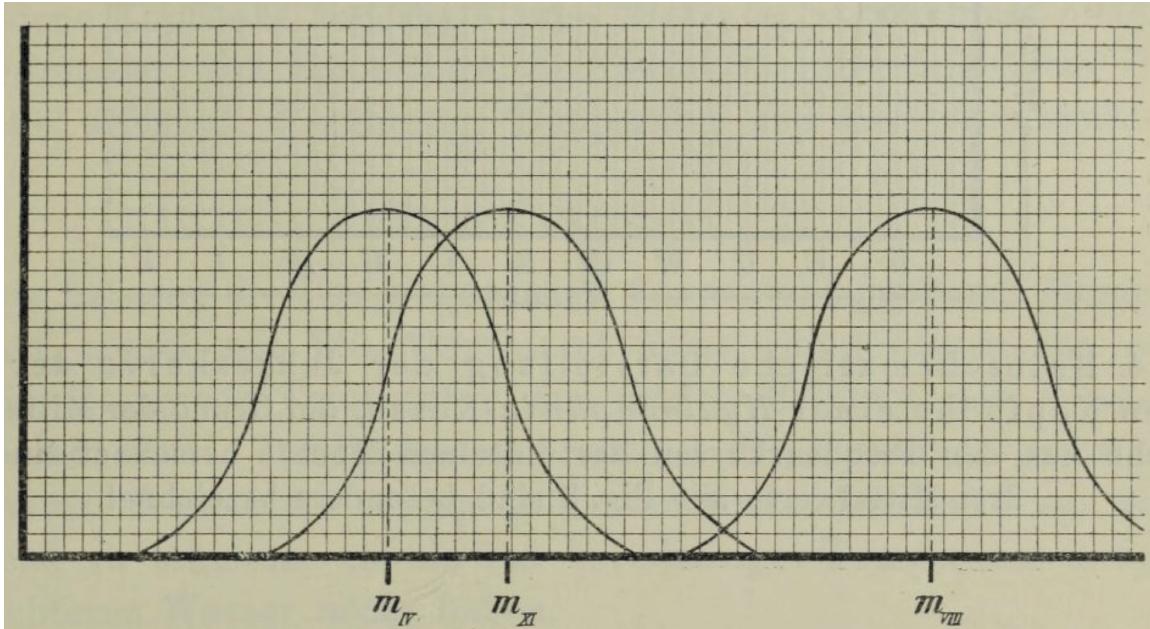


Fig. 3. Semi-schematic representation of three frequency curves of relative head-height of certain *Hyalodaphnia* races collected from the wild. The horizontal axis represents a measure of relative head-height, and the vertical axis represents the percentage of individuals with a given head-height. The left curve represents grown April-caught ♀♀, the right August-caught, and the middle November-caught. For each of the three curves, the mean is indicated with m_{IV} , m_{VIII} , and m_{XII} , in turn indicating the number of the month.

with glycerin or salt. Considering that the result was always negative (apart from a definite degeneration of the older cultures in higher concentrations), it would not be profitable to further explore the details of these experiments.

Increases and decreases of the temperature itself are in contrast to these results, as Wo. Ostwald very rightly observes (though he does not interpret them properly), showing a noticeable influence on the head-height, in addition to an effect on overall body size.

We shall consider the latter, simpler alteration first. It is known that body size can in fact be reduced by heat, but only when holding constant a poor to middling diet. With more food, in contrast body size is absolutely not reduced by heat, but rather considerably increased.

The connection here is obvious: under higher temperatures bodies require a larger amount of food for their higher vital activity (movement, heartbeat, and fertility are very obviously increased). Therefore, they must devote less material to their growth, with only a meager quantity of food at their disposal. If in contrast food is available in surplus (which is unlikely to be the case for the pelagic types, which sustain themselves upon all available detritus, Bacteria, *et cetera*), then warm conditions induce an increased capacity for assimilation and with it an increase in body size.

The same principle applies also for head-height or the "Helm" of the animal, as shown in numerous important experiments upon this important point using *Daphnia longispina*, *galeata*, and *cucullata*. I will not go into these

experiments in detail, and only share that I have achieved greater head-heights in my cultures with low temperatures and rich diets of *Chlorella* than I have with high temperature and poor diets. Specifically, after a week-long incubation in a thermostat at 30°, the heads stay small if there is not a rich algae diet (consider that the "natural" temperature ranges from 18-25°). Even at 6-10°, I can achieve higher heads with rich food than I can with lesser nourishment at 30°. At temperatures under 5° or above about 32°(the bounds differ between biotypes) metabolism is quite poor, such that past these points animals 'stunt' or die.

The smaller heads that overwintering *Daphnia longispina* or *cucullata* show in the colder seasons (Wesenberg-Lund was the first to show that they are all alike) are attributable on the one hand to lesser food, and on the other to a degradation of the assimilation capacity by cold. The high helmets of the summer, however, are achieved through increased food; or more exactly, through the consequently increased assimilation. (Concerning the other innate factors effecting helmet size, compare page 143 below).

The subject presented here is too complicated to be immediately handled in detail. What we need immediately, however, we can abstract: the most essential factor of the 'environment', the living situation of our *Daphnia*. It is particularly important that the quantitative differences of the assimilation be directly proportional to the quantitative differences of tested characters, while a second trait (sex, page 144) is held proportionally constant.

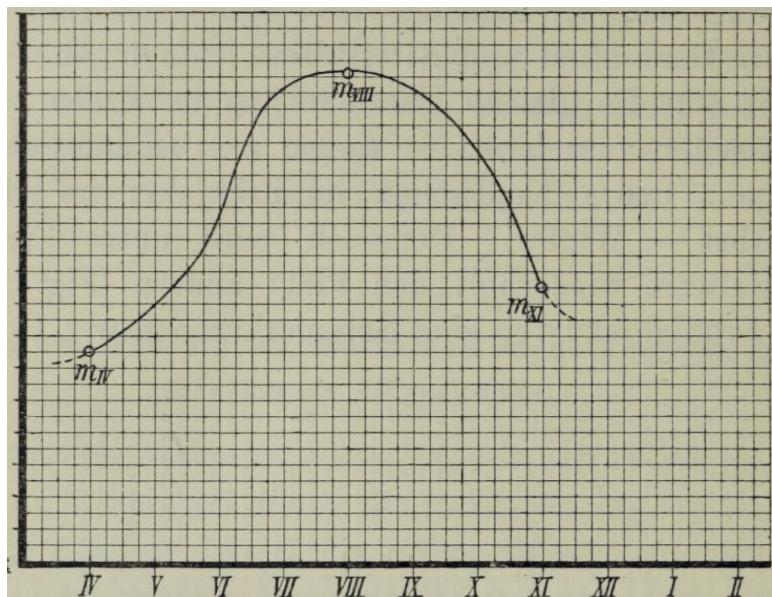


Fig. 4. Semi-schematic representation of the change in relative head-height of a *Hyalodaphnia* race, based on means calculated from the frequency curve of each month. The vertical axis represents a relative head-height measure, the horizontal access indicates month of the year (as numbered in Roman numerals). Three means from Fig. 3 are indicated.

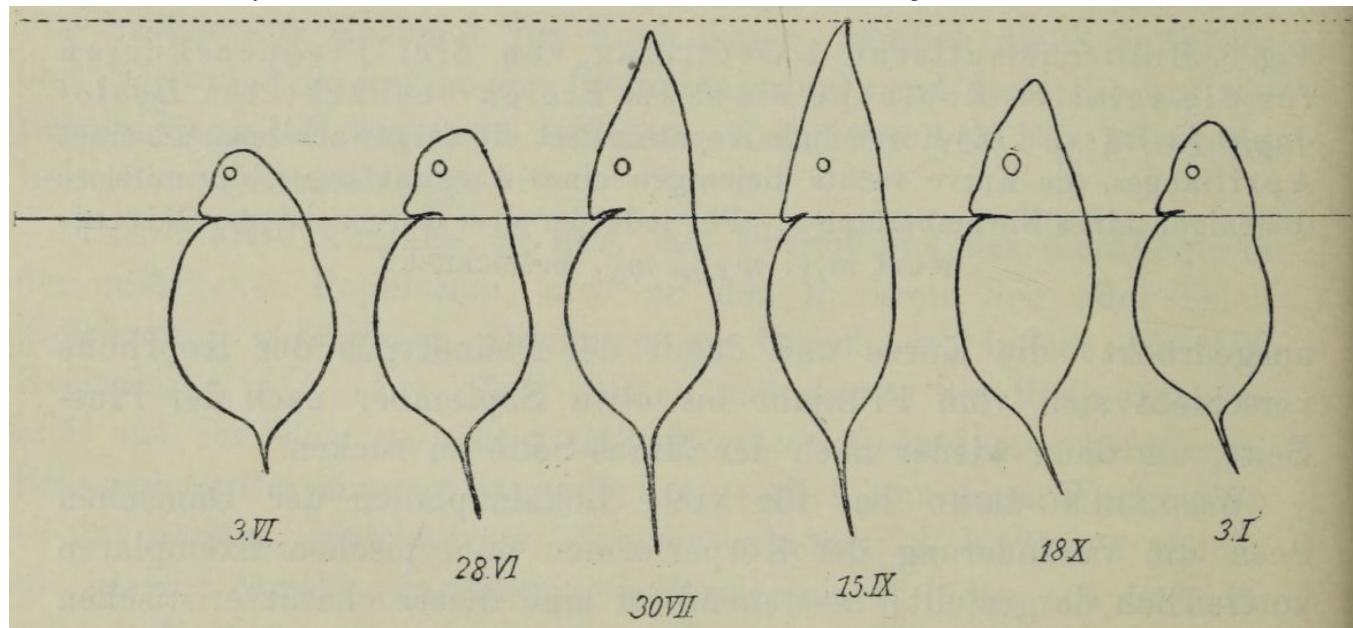


Fig. 5. Representation of the cyclomorphosis of a *Hyalodaphnia*, after Wesenberg-Lund. These are likely single typical examples of large similar populations, drawn next to each other. (By joining the vertex points one may obtain a year-curve of absolute head-heights, which however is not so useful as the means of relative head-height used in Fig. 4. The examples used by W.-L. for illustration (Plankton-investig. of the Danish Lakes II. 1908) from Skanderborg Lake were chosen because they showed a definite periodicity in both spine-length and spine-angle across months.)

It is methodologically important that the influence of temperature, the second factor affecting assimilation intensity, be easily disabled in analytical experiments. For this, one may simply take care for the warming of the cultures. The same may be said of the total state of the 'environment' of the cultures: salt and gas content, volume, and light in adequate and equal amounts, such that the single factor of food supply is left, and this last is well-measured (see appendix 1, page 166).

We can thus establish the supplied quantity of food as the measurable cause of head-height particularly. Similarly, we can ignore the quality thereof if we feed comparable cultures with the same pure cultures of *Chlorella* (or some other algae) in measured amounts.⁸

Innate factors We must now put aside nutrition (and the secondary factors of temperature and so on, that we have eliminated through experimental treatments) to take another variable factor into consideration, namely the influence of the generation number. This factor is a part of the residual constantly-working heritable helmet-potential, whose existence we will shortly observe in total. We saw above that assimilation intensity (as a product of nutrition and temperature) must be particularly crucial in reshaping both the body plan and the head height. From this we may clearly understand this special assimilation process, and we might say that this operation of nutrition on the helm-substance is directed by inner forces, which are in truth the decisive factor. This is, namely, the inherited potential for helm-growth. We can understand this potential as acting on available materials in the hypodermal cells of the head; or better, as an arrangement of these materials, which reacts to the certain qualities and quantities of nutrition (blood) through the construction of a ridge-like anterior process of a certain height.

We now approach what seems to me an especially important point, so it will be beneficial to briefly consider the morphological and physiological components of this helm-growth. The critical region of the Daphnia head is normally found by comparison to the chitin-covered hypodermis, which is thickened on the rest of the body. When no helm is present, this has something of the form of a roof. Invariably (except in the case of very low heads) this roof shows a sharp ridge; the systematists say that the head is 'keeled'.

The thickened hypodermis underlying this ridge or keel is the seat of the 'helm-potential', whose nature we would like to investigate. The hypodermis can remain low and compact throughout life (as shown by Fig. 6a), it can however also grow out to a high and narrow protrusion (Fig. 6b), which then is covered with a thin layer of chitin and takes on the

⁸Unfortunately it is often not possible to perform immediately consecutive experiments. This makes it rather uncomfortably noticeable that nutritive value of these algae fluctuates in ways that are difficult to understand (perhaps together with their variable tendency to stay suspended in water or sink to the floor). Especially, care must be taken that food algae must not be grown next to Bacteria or other algae, which often (for instance with the spiked Raphidia) render the algae unpalatable to Daphnia. In such cases one may obtain totally untrustworthy results.

appearance of a helmet when viewed from the side.

In reality the 'helm' is a narrow blade; the young animals in the mother's brood compartment maintain it, so long as their chitin is not yet hardened; in such close spaces it resembles a sleeping cap turned down to the left or the right.

But how does the stimulus direct development at this point of helm-forming potential? This may occur by two quite different ways, depending whether it acts upon the helm-building cells of an embryo or of an adult animal. Namely, in the latter case, this cell mass retains the potential for development throughout life (or at least until the 'advanced age' of 10-12 weeks). In younger animals the reaction of course proceeds much faster and more completely, than in the case of older examples.

In the embryos the sustenance that mediates the stimulus approaches the growing cells by means of an external source; this is the nutritive juice in the brood chamber of the mother. Higher or lower concentrations of this stuff is that which chiefly determines whether the young animal receives a high or low head. This juice is familiarly thought to exude from a cell layer on the floor of the brood chamber, where it itself is obtained from the relevant nutritive pool of the mother's blood. Additionally, the yolk content of the embryo appears to contribute to embryonic or youthful helm formation.

In the second case, of the formation of the helm before the molt of the grown animal, it is the greater or less nutritive content of the blood plasma that acts as a regulating stimulus. Furthermore, the activity of amoeboid cells comes into play, which are found at the locus of helm development (and also at the base of the mantle spike) in the form of a small plaque. This is likely composed of 'blood cells'; their activity can tentatively be identified with the influence of the blood upon the helm or spike development. See Fig. 7.

Unfortunately the processes of assimilation are researched little or not at all in these Entomostraca⁹ (see part 2, S. 168). One may however learn something of this process by means of the complete transparency of these animals. If given rich food, a heretofore malnourished Daphnia will very quickly begin to show characteristic alterations. The gut will immediately fill with algae, and one can then measure the time until the appearance of the resorption droplets appearing in all of the gut cells. Then follows a cumulative alteration of the heretofore unseen (because totally water-clear) plasma into a visible juice, at first yellow and then gradually rather opaque. From this blood juice the whole body is provided with a rich stream of nutrition. In a surprisingly short time (at permissive temperatures), one now observes the previously invisible gonad swell, and the previously nearly fatless body becomes fat, appearing with its characteristic red or violet color. The action of the enriched bloodstream on the helm-developing cells is similar: the cell layer increases through imbibition and perhaps also increased number of cells, and by the next molt the head height shows a substantial increase. Conversely, one can decrease the head-height through a molt by starvation; this has been described by Wesenberg and others. Even for some time be-

⁹trans.: an outdated taxonomic category of crustaceans.

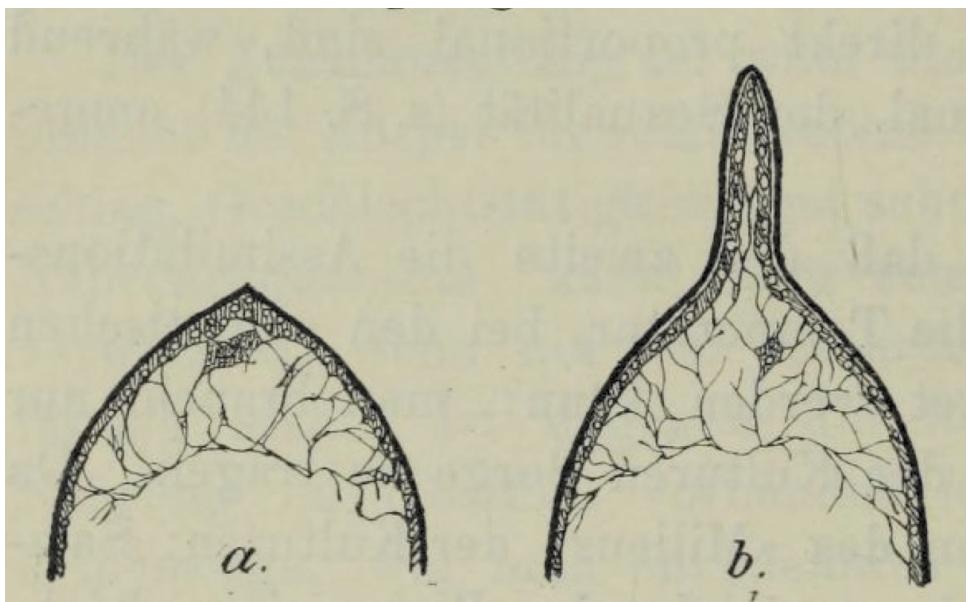


Fig. 6. Two frontal sections through the crown of a *Hyalodaphnia*, conducted approximately in the plane of the 'long axis'. Chitin in black, hypodermis cross-hatched with white inset kernels. (Underneath branched connective tissue single large amoeboid cells is shown.) *a*, Helmless animal (as Fig. 5 at left); *b*, Beginning helm growth.

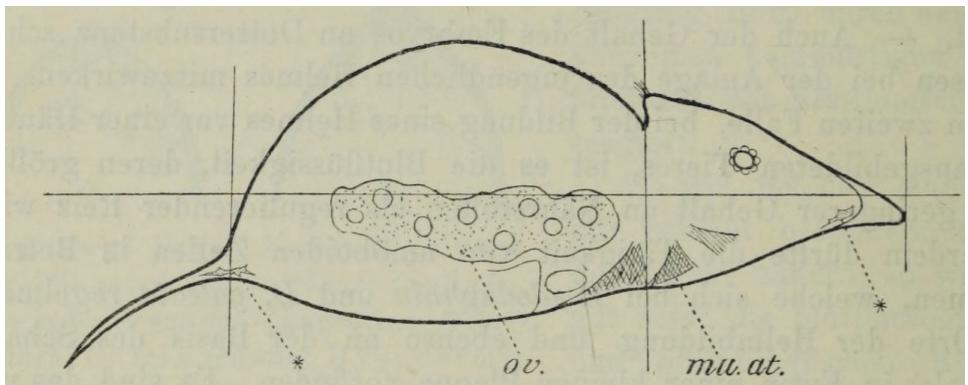


Fig. 7. *Hyalodaphnia*-♀ shortly before molting, with diminished nutrition (or assimilation). The contour of the new helm is already visible, it is already become substantially diminished. * indicates plaques of amoeboid cells (blood cells?), which play a role in helm and spike development. *ov* ovary. (Compare also the explanation of Fig. 1.)

fore the molting, the new smaller helmet Anlage is embedded in the earlier one, such that the two contours are observably separated (Fig. 7).

We can finally state that, at a given temperature, each quantity of food corresponds to a specific increase of the head height, even as it corresponds to the building-up of fat bodies and ovaries. For each biotype, there are set rules for the allocation of available surplus (as absorbed and dissolved in the blood) to each different region or organ of the body, and to the increase of energy production (heart rate, swimming motion). For example, there is pronounced competition between the ovaries and the helm-building cells. In each case, the severity of the ‘war’ between ovary and helm depend upon many aspects of the assimilation process (quality of food, temperature, etc.), on the special character of the biotype in question, and most importantly on the generation number.

In the ‘generation number’ we have a ‘cyclical variable’, which is to say specifically, a successively diminishing role of an innate factor in helm development. This inner factor, the ‘helm-potential’, reacts in different generations with different intensities to the same fixed environment. By this I mean nourishment and temperature and therefore assimilation, which we previously learned to be the regulating causes or regulating stimulus of helm development. I noticed that the first generation reached a markedly smaller average head-height compared to later generations, across many forms of *Hyalodaphnia cucullata* with similar nourishment etc. Further, for two local biotypes of the same species I can state that the middle generations reach a higher head-height than the later, in a similar environment.

Here it is worth noting that the early and late generations of these elementary types normally occur in the spring and in autumn, when in the wild they can develop only an inferior assimilation intensity relative to those in the summer. Thus other factors of assimilation are noticeable, in addition to the regulation of nourishment quantity by temperature.

With these, we have learned the innate factors of helm development: the innate cyclically diminishing helm-potential over generations; and on the other side the external factors: first nourishment, second temperature, and further the gas and salt content and other qualities of the water. From all these various external factors results the dramatic variability of assimilation intensity, to which we have learned to attribute the similarly variable head-height. Otherwise expressed: the assimilation intensity acts as a graded stimulus, that releases a corresponding intensity of reaction in the substrate of the helm-building cells. This reaction leads to specific heritable helm-potential in this same substrate.

b. How do we determine the collected phenotypes of head-height for an elementary type, and how do we represent them?

We desire to study the development, inheritance, and changing of these specific helm-potentials. From these studies, we desire to see whether the position occupied by De Vries and Johannsen — change of the quantitative trait only through mutation and determination by the environment — is supported or not. Through our knowledge of the helm-building

factors, we are now in a position to make determinations regarding the causes (via induction or regulation) of all possible head-heights independent of each phenotype that this character depends on, for each elementary type that we investigate.

Without giving all the figures independently that were found for different biotypes, we wish to briefly consider how one may conduct an analysis of the phenotypic entirety¹⁰ of a character. Further, how may one best represent this analysis to lead to a solution of the question we have posed?

To simplify this general representation, we confine ourselves to the triggering cause (assimilation intensity) related to the factor of nutrition, and attempt to isolate the graded effects of this factor for study. We will leave aside the lesser causes of temperature, light, and water quality, by considering only cultures in which these factors are held constant and only the quantity of food is varied. (A study of such cultures in which nutrition is constant but other factors, such as temperature, are held constant, would not be novel. Rather, it would arrive at the same goal by an alternate path.)

Moreover, we observe only the effects on ♀♀ and leave ♂♂ alone. Finally, we restrict ourselves to only three levels of nutrition: poor, middling, and rich. Our task is to determine the phenotype of head-height for each such level.

To achieve precise nutritional quantities, we use carefully filtered water with certain quantities of *Chlorella vulgaris* (or similar algae: Palmellaceae *Kirchneriella lunaris* or others) added back from pure culture. We must replenish their quantities daily to avoid depletion by transferring the animals, because otherwise *Daphnia* will reproduce due to favorable conditions, and likewise, algae will fail to reproduce under unfavorable conditions. This redeployment of animals can be effected with a pipette, or for large cultures it may easily (and with practice speedily) be carried out with silk gauze. For “poor nutrition” conditions, one provides enough algae to sustain life, and also to allow reproduction; though only one or two eggs are desirable. The “rich nutrition” is attained by supplying algae in excess, and is immediately recognizable by the green color of the cultures. In this greening and the overabundance it signifies, one still finds impairment of some *Daphnia* (decrease of head-height and general vitality). Otherwise, in this thick, green-yellow algae water do well, and it is only here that the maximum head-height, body size, and speed of movement are found. (More curiously one sees such natural forms in the animals that live in pelagic zones of clear lakes, for instance *D. longispina* of the Lunzer Untersee). “Middle nourishment” lies in between the empirically determined minimal and optimal quantities (“poor” and “rich”). The correct input of algae is somewhat subjective, and is assisted by the quantity of *Daphnia* in culture, and is therefore a retrospective designation following experiments considering these factors. The method by which one may know the phenotype or mean value of a character for a certain life stage is well-known enough. One

¹⁰The ‘collected phenotype’ (*Gesamtphenotypus*) of a character we may unfortunately not say, because Johannsen has defined this term as the sum across characters of an organism, for instance their average in some particular condition.

uses a large number of measured individuals to construct a frequency curve ("Galton curve") or, as is simpler in some cases, a distribution schema. From one or the other of these one reckons the mean value of the relevant character.

This mean value expresses the following: for the collected individuals of the relevant li in these cultures, which are essentially identical (see the first appendix), the "normal" nourishment of the eggs and embryos in the mother animal¹¹. The higher variations owe their higher head above all to accidental chance, that an abnormally small number of eggs fully matured in the given environment. The lower variations owe their deviations similarly to too great egg production all at once. However, generally the egg production of age-matched ♀♀ raised on similar environments are rather constant. The middling deviation (variability index) is here in most cases not meaningful, because in this case it gives large differences and complications even within the same Biotype, which we must prefer to neglect. Figure 8 shown here represents no specific instance, but rather a schema for the head-height frequency curves that we obtain for *Hyalodaphnia*.

We can draw the three helm-height frequency curves for poor, middling, and rich nourishment in the same coordinate system (Fig. 8), and with them the three corresponding mean values m_1, m_2, m_3 , the three phenotypes.

In overview, let us once more represent the dependency of the same phenotypes on the conditions of life (nourishment) through the following graphical representation. I hold on the abscissa axes the nourishment quantity, from zero on the left to overabundance on the right. As the assessed value, one may use either 1 cbmm¹² of an intensely green Chlorella nutrient solution or 1 small platinum loop full of Chlorella from an agar culture. Both quantities are agitated in a certain quantity of water (that differs between species). Mathematical exactitude in measuring this dose is obviously difficult, and unnecessary.

From this abscissa axis I have raised to each single point an ordinate, which represents the mean value of head-height derived from the frequency curve, to which the nourishment quantities correspond. [Trans. note: This is confusingly phrased. In modern parlance, Woltereck has created a density plot. In his time, this was not so common a procedure, perhaps leading to confusion. Alternately, I may have missed his meaning.] When I connect the end-points of the ordinate, I obtain a curve (Fig. 9, 12) which I will describe as the "Phenotype curve". The curve constructed thus can be applied to some quality of the water (temperature etc.) that is shared between all cultures.

If I want to represent all the phenotypes of the analyzed character, I must next fill in all the relevant temperatures in my coordinate system of the phenotype curve, or at the for low, middle, and high temperatures. Through this I would obtain three phenotype curves of the same character for each elementary type, the ratio of whose positions against one another will be somewhat different, as determined by heritably

¹¹trans. note: note that in this case "normal" environment' is not a synonym for "Gaussian".

¹²Trans. note: probably cubic millimeter

fixed differences between Biotypes.

Further, I must distinguish between the first, middle, and late generations (computed from dormancy onwards) for some elementary types. The form and the relative positional relationships of these curves are also heritably fixed for each Biotype.

Upon such analysis, the different generations of some Biotypes not only show different reactions to the provided food quantities, but also to temperature differences while holding food constant. It is therefore necessary to consider three temperature curves for each generation.

Finally, the curves for the helm-height of the ♀♀ and the often quite different ♂♂ animals are actually separated from each other. The ♂♂ animals of two elementary types can hold themselves totally apart in these points, whereas the ♀♀ animals show great similarity (for example the colonies from Frederiksborg and Moritzburg).

However, to attain a tolerably complete representation of the phenotype regularities, it would "suffice" for such a quantitative character as head-height to fill in the already not-insubstantial count of nine phenotype curves, namely for the control of the ♀♀ in the following combinations:¹³

Beginning generations " " " " "

Middle generations " " " " "

Late " " " " "

Now, each phenotype curve of the analyzed characters is based upon the mean values of at least three frequency curves, for poor, middle, and rich nourishment, so for approximate knowledge of the specific reactions of this single character we must use no fewer than $3 \times 9 = 27$ cultures and from them obtain frequency curves and their related middle values.

We must here neglect to go through the particularities of the number relations. In this instance, it comes down to clarifying the coming into being of such quantitative characters as follows: the specific head-height of a given elementary type is heritably controlled through a large number of relations.

Even with these 27 related values, we remain quite far from an exhaustive definition of the specific helm-height and helm-potential of a tested elementary type. On the one hand we have left out all the intermediate values between the three chosen values for each of the helm-building factors. On the other hand, we have left out specific differences due to external factors, gas and salt content of the water etc., and also the effect of intrinsic regularities. Examples of such intrinsic regularities include the correlation between helm-height and germ cell proliferation and the development of head-height from molt to molt. We may file also with these factors other particularities of the apparently simple head-height and its heritable basis.

Now, overall we have very little reason to see head-height as especially complicated in the regularities of its relationship to environment relative to other bodily organs. A more exact analysis of each quantitative character may yield a similar richness. The analysis of "helm-potential" is merely

¹³The following orthography follows Woltereck.

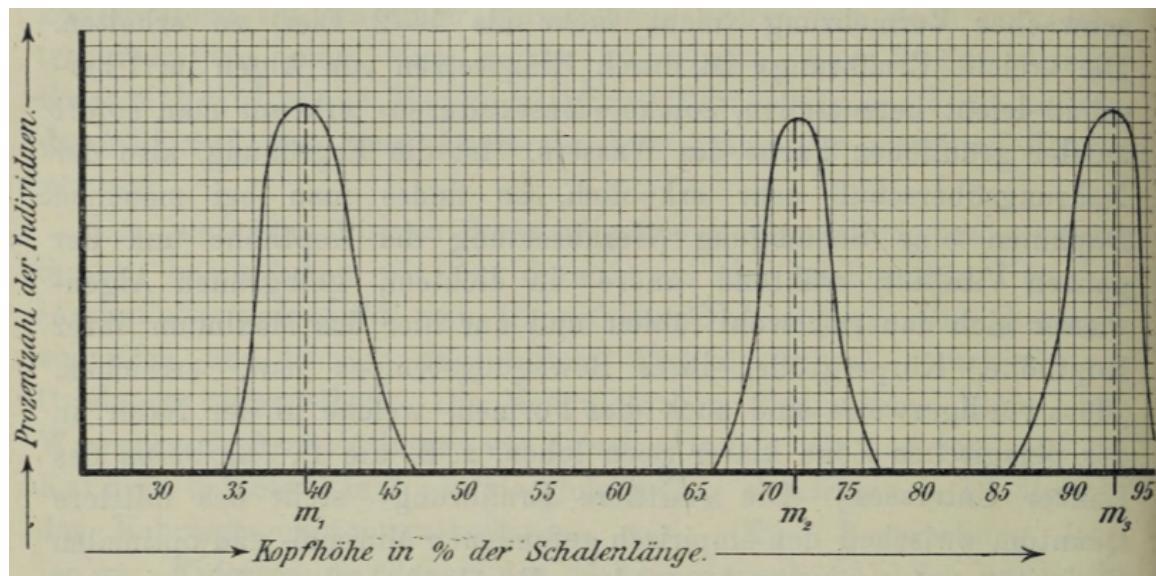


Fig. 8. Semi-schematic representation of the frequency curves for the relative head-height of a *Hyalodaphnia*-race, under minimal, middling, and optimal nourishment conditions, with the corresponding mean values: m_1 , m_2 , m_3 . Abscissa label: Head-height as a percentage of the mantle length. Ordinate label: Percentage count of individuals.

more readily measurable to prosecute than the peculiar reaction intensities of the burgeoning of the ovaries or the fat bodies, the eyes, and so on. (At the least, we will later (s. 144) approach "sexuality" more closely, as an entirely different sort of character in its corresponding relations.)

We have hardly any reason to expect a principally different nature of quantitative character potentials in other organisms. For example the length of beans in Johannsen's biotypes would be such a character, whose potential (gene) would be found to be related to specific relations of the environment, even if we manage to test the single influences of its circumstance (as in *Daphnia*). Regarding the quantitative traits (coloration etc.) whose specific differences appear much simpler to the casual eye than those described here, I will not venture anything. Different sides of the argument have already confirmed that they cannot be principally different from the quantitative traits and potentials.

And so, what have we achieved so far?

The understanding that any such quantitative character in its wholeness can only be fully understood and characterized by a great horde of relations (i.e. the relationships of reaction intensities to trait variations from stimulus intensity to trait variations from nutrient assimilation). The whole of these relations may be represented only through a number of data tables, or more comprehensively through the 'phenotype curves' that I have put forward for this purpose. If we want to realistically analyze the importance of these various specific relations, the number of these curves is nine for our cursory evaluation here, and will grow to an endless quantity from there. The totality of these relations, described in these curves, we can call the **reaction norm** of the quantitative character in question. We can quiet this cacophony somewhat to the slighter number of phenotype curves we

actually have, which in praxi we must do. Many times over we will have to decide to construct a phenotype curve only for the "middle" generation and a "middle" temperature. We can then also from these curves speak of the "partial" reaction norm (Fig. 9) of the corresponding character. We may however go no further in our claims in these cases: we cannot take as given any measure of a quantitative character for which for which we have not at the least measured the same (in this case, middle) grade of nourishment (or assimilation).

c. What can we infer from the genotypical basis of a specific head-height and the genotypical difference of this character for two elementary characters?

The foregoing illuminates that we can define or show support for an elementary type only through the specific and relative reaction norms of its different characters.

We interest ourselves in these facts not only because of their consequences for the systematics of plants and animals, but rather principally for their import for the sciences of heredity and speciation. To familiarize ourselves with this importance, we must recall two indubitable and eternal truths. First is the proposition that everything that is specific to an elementary type or a variety, by which I mean that which distinguishes it from other biotypes, must be bequeathed to the next generation in the germ cells. Second: a new type or elementary type arises through the heritable alteration of any specific character.

The "genotype" (the genotypical foundation) of a quantitative trait is its heritable reaction norm. From both of these propositions the following is very clear:

1. The total reaction norm with all its countless specific

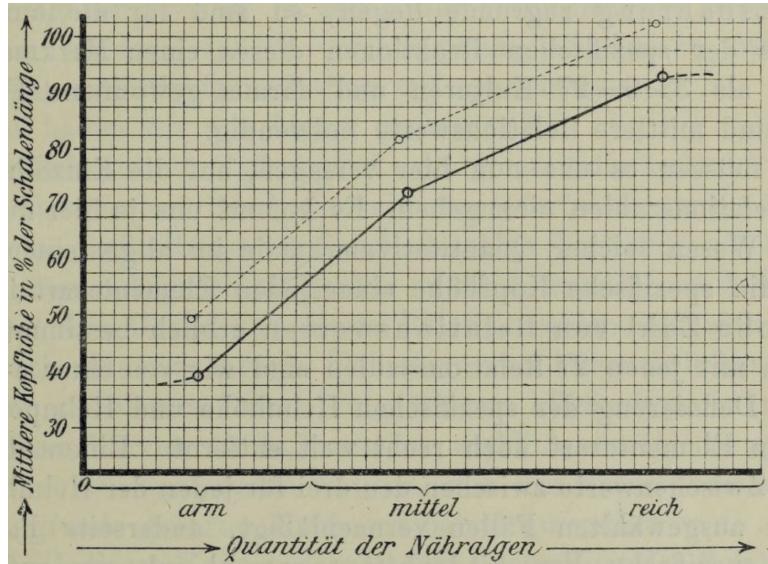


Fig. 9. The three mean values of Fig. 8 represented as the simplest “phenotype curve” (black lines). The animals represented belong to a middle generation, cultured in middle temperature.

relations is inherited as the principle for helm development (helm-Anlage). Therefore, the “genotypical basis” or “genotype” or also the “gene” (all terms from Johannsen, the appropriateness of which we will not speak of here) for helm development are all equivalent to the reaction norm, as we have defined it.

2. The biotypes of *Daphnia* arise and have arisen through heritable changes in the reaction norms of their head-height and their other characters. From these reaction norms the natural question arises concerning our assumptions: are these reaction norms changed only through mutation and without determining influence of the environment? Or are they continuously modified through the action of changing environments?

We can posit a discrete modification of helm development (if such actually exists distinct from the more familiar continuous variation) only under like conditions of the environment, and specifically only if in similar conditions of nourishment the head-height appears by some quantity greater or lesser in one type. Such a difference transgresses the boundaries originating in embryonic nutrition and resulting in the continuous frequency curve of the phenotype. Such modifications can in fact occur; I have observed them myself for the rostrum length of *Daphnia longispina* (Fig. 10). In all circumstances, such mutations naturally assert approximately the same difference of their mean from that of the original form. A comparison of the phenotype curves constructed for each form corresponds somewhat to the picture in Fig. 11. (We can confine ourselves in the following to the reaction norms expressed in phenotype curves for a middle generation and a middle temperature.) For the case represented in this picture, both propositions of the mutation teaching are in effect:

The difference between biotypes is

1. discrete (I have also observed that half-races after the style of De Vries have heritable differences in rostrum length that do not cross over), and
2. not determined by the environment (because the difference develops equally at all grades of the environment, and can be attributed to no particular condition of the environment. Rather, the trait develops to its highest value through the joint action of both environment and the difference in the heritable stuff.)

These typical features of mutations can be dismissed no more in our *Daphnia* than in higher plants (De Vries and others), beetles (Tower), or vertebrates (Arenander and others).

The question is merely whether mutations can be ascribed a general and exclusive role in modification of types. And, as I show by means of the quantitative characters of *Daphnia*, this question can be with certainty answered “no”.

First, to dwell upon helm-height, the phenotype curves of two elementary types (which through their various conditions of growth become overlapping) must show a distinctive relationship such as shown in Fig. 11 if they are derived through mutation. There must therefore exist in each life condition a material and approximately constant difference between the two types.

This is how Johannsen understands his biotypes: these differences shown by two phenotypes in a common life history are the mutationally derived differences, and therefore reveal the differences between the genotypes of his elementary types. These genotypes are determined and stable quantities that must maintain the expected noticeable difference, as schematized in Fig. 11.

The beginnings of genotypical distinction: partially distinct reaction norms. — Natural Transitions. With my

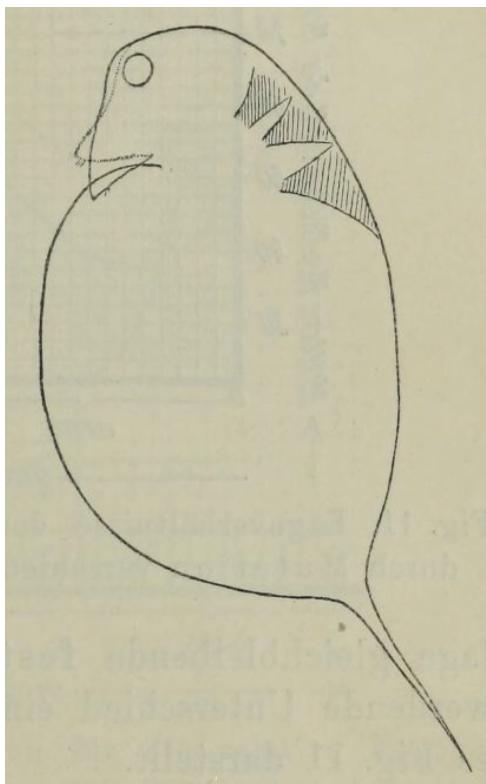


Fig. 10. A heritable, discrete recurrent variation of the rostrum length of *Daphnia longispina* (Lunz Untersee). The original contour of the rostrum is drawn in dotted lines.

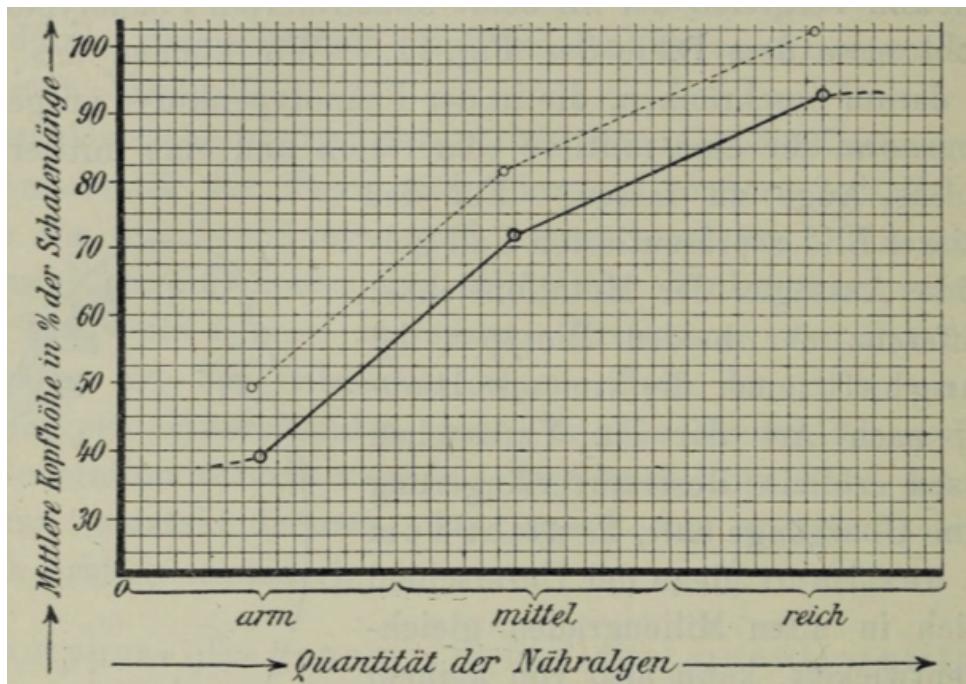


Fig. 11. A schematic: relations of phenotype curves of a character for two elementary types diverged through mutation. [trans.: note the strong resemblance to Fig. 9. It is not clear whether the similarity is intentional or an editorial mistake; in any case the figure is not obviously inappropriate in either case, and may simply constitute thriftiness of effort.]

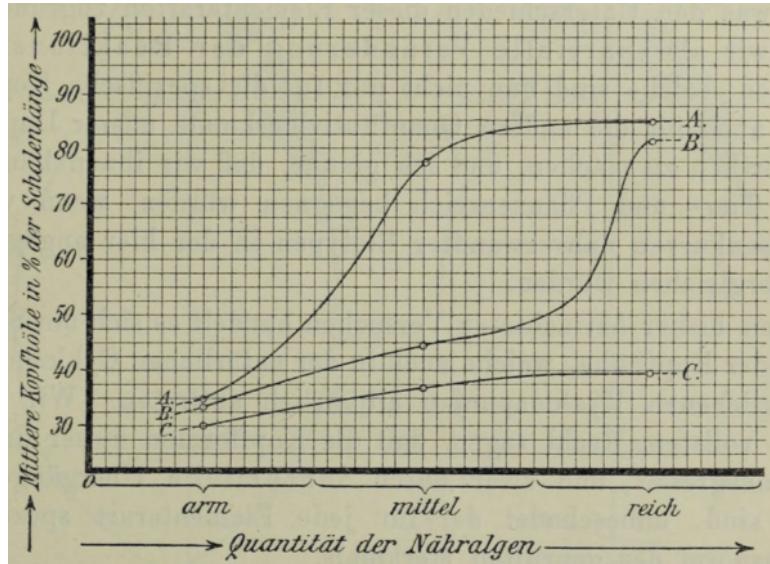


Fig. 12. Relations of the phenotype curvers for the relative head-height of three elementary types of *Hyalodaphnia cucullata* (♀♀). A, Local type of the Moritzburg lake; B, the like from Borsdorf; C, the like from Kospuden. All curves show values for middle generations and constant middling temperatures.

search for the possibly small but heritable differences between *Hyalodaphnia* landraces (and also after the beginnings of type divergence among such animals), I found also differences in reaction norms. That is, differences in the relative position of the phenotype curves which in no way fit the expected schema laid out above for mutations. Fig. 12 represents phenotypic curves of 3 local forms of *Hyalodaphnia cucullata* collected from middle generations raised at a middling temperature (18–20°). The animals in question are so similar in their head-height that I had great difficulty distinguishing them from one another under some conditions. This shows first that the three landraces are all nearly identical for the phenotype of head-height under low feeding (round, short heads), while for middling sustenance a sharp difference appears.

One elementary type (from Moritzburg, appearing similar to the animals from the Trentsee¹⁴ and Frederiksborg lake) already has a great head with middling nourishment, while with like algal quantities the helm of the second elementary type (from Borsdorf) remains minimal and third (from Kospuden) has hardly any helm to speak of.

Further: the head of this last form is only slightly taller with rich food, while the Moritzburg and Borsdorf types develop very similarly tall helms with generous nourishment. These last two biotypes show similar forms at both lower and higher assimilation intensities, while they react quite differently to a middling intensity.

This behavior of the phenotype curves forms a characteristic contrast to the schematic curves presented for mutations (Fig. 11). The quantitative differences between curves are to be sure genotypical and heritably determined, but this

difference is even more certainly not abrupt and derived independently from the environment, because for each comparison it can confer large differences (for middle nourishment quantity) and also can completely erase a difference (as for weaker and stronger nourishment conditions). By the same token, the response cannot act through a singular regular difference between two fixed genotype values, because the difference due to environment runs the whole spectrum from nil to full helm development, from which we can in no way determine a fixed value across nourishment conditions. Finally, this difference must surely be caused by the environment factors (compare to appendix 3), as has been shown here through the precisely controlled conditions of the environment.

We can label that which underlies the differences between these elementary types the “partial alteration of the reaction norm”. These are not specific to head-height, but can also be held accountable for other quantitative characters of our *Daphnia*, and I believe that we will frequently enough encounter them in the plant and animal kingdoms if we would compare the phenotype curves of nearly-related biotypes in the fashion I describe here.

The experiments reported so far concerned the variations of head-height, which also occur annually in the natural cyclomorphosis of the compared *Hyalodaphnia*. We can also say with the greatest certainty that the head forms of these three biotypes are transgressive, and overlap by means of “natural transitions”, regardless of the character reaction norms specific to each elementary type.

The evidence that the *Daphnia* local forms are differentiated by means of continuous environmentally-determined alterations points to additional paths for inquiry. Next it leads to a more exact study of the transitional forms using manipulations in culture.

¹⁴For this living material from the Trentsee I thank the generosity of Prof. Zacharias (Ploen).

Artificial transitions, in accord with the natural relations of the environment. In my lecture of the previous year I showed head-height, spine length, and also "spine-angle" and "brow-angle", for two local elementary types of *Daphnia longispina* (Fig. 13), which can indeed not be compared in their conditions of environment, except through the application of a dramatically changed life history. That is, the Untersee-*Daphnia* live in Nature sparingly and the Obersee-*Daphnia* live with substantially richer food. When I give the Untersee-*Daphnia* an overabundantly rich diet they become closer to the Obersee-form, and when I give them a very poor diet (which is to say that I starve them), the Obersee-*Daphnia* come to resemble the Untersee-form.

In this fashion a continuous series of transitions appears between the two elementary types with relation to a single character. These transitions show 1. that the characters of the two types are not made different by the discontinuous means of mutation, and 2. that these differences must be a function of the peculiarities of the environment.

Through persistently rich environmental conditions in the Obersee a natural "fat form" arises, and likewise through poor conditions in the Untersee we observe a natural "hunger form". Both forms are now heritably determined, such that under similar conditions their difference is fully preserved, even under exchanges, as long as the conditions used are those that we observe in nature. That is, as long as we use the conditions of the sites from which these types originate. (Therefore these forms are in no way "site modifications", in that they already must be made alike by similarity of the environments.) [trans. note: ??? colloquialisms a little tangled for me]

Only if we test the reaction of the character to extreme environmental insults (conditions not encountered in nature) do we find extensive similarity of the reaction intensities between the two types. Therefore when we compare the "phenotype curves", for example for head-height of the two elementary types, then we obtain a similar picture, like what we saw with the "natural" environmental curves A and B of *Hyalodaphnia* (Fig. 12): strong differences in the middling region of environment, but similarity in the environmental extremes.

To make the behavior of the aforementioned *Daphnia longispina* biotypes very clear, I have constructed the phenotype curves here (instead of the distribution curves I used in my publication of last year), because these frequency curves allow a better comparison for *Hyalodaphnia* diagnosis. I believe that the appropriateness of this step is obvious.

We may take this case as an example of such situations, that even if we cannot find "natural transitions" bringing types into phenotypic similarity, like the *Daphnia* of the curves A and B (Fig. 12), we can nonetheless identify "artificial transitions" through extreme conditions beyond the scope of natural environmental differences.

This last case we can label as the second step of biotype divergence (curves A and B would then be the first step). A third grade of divergence would be reached further in the cases represented by phenotype curves A and C. Here the reaction norm of head-height is entirely different, while in the first two cases it is only partially different. We can hardly go

wrong in the assumption that the temporal ordering of these divergence stages corresponds to the rules of this schema, at least for quantitative differences. As a preliminary stage of our first divergence stage (curves A and B) we observe an ultimately false site modification, whose phenotype curves remain identical in nature.

Different reactions of successive generations agree with their natural environmental conditions. Finally, I ask the reader to recall that for certain elementary types, the reactions norms differ between generations, and thus we represent the phenotype curves for early, middle, and late generations differently in our analytical representation (S. 134).

This previously presented fact stands as further evidence that the potential for helm growth cannot be seen as a unitary measure, which cannot be altered by discontinuous single modifications. Also in these generational differences there are even more dramatic transitions into the behavior of other biotypes, such that we do not require further transitions to recognize the types as continuously rather than discretely differentiated.

However, we clearly see something different in the behavior of the successive generations: the first generation after dauer produces a lesser head than the middle generation under similar nourishment. We can know this certainly in the cultures, to which we can allow any quantity of nourishment we desire and simply supply like amounts. For this behavior it is difficult to find another explanation, except that the March-April-May generations ordinarily experience a poorer nourishment in their waters, probably much poorer, than the later July-August-September-born animals. This regular inferior nourishment of the first generations seem to likewise show their traces in the total potential of helm-height, as the altogether poorer nourishment of the Untersee colonies have. In both cases, this results in a heritably fixed diminution or hardening of helm-building in those generations and likewise those landraces in which helm-building has long been out of use.

In this interpretation we make the assumption that the higher heads in these special cases are ancestral. However, we can nearly take it as given that the regularly richer nourishment of the summer generations and the more richly-treated colonies has gradually caused these generations or colonies has led the helm-building reaction to proceed more easily and with greater intensity. For our theoretical purposes these two statements are not materially different; the sleeping agency of a particular environmental condition has gradually and heritably fixed the corresponding grade of reaction (see Appendix III).

Also the late generations of a landrace that normally arise in the autumn show a decline in their helm-building ability compared to the middle generation not only in the field, but also in my cultures under like conditions of nourishment. These generations show a pattern of behavior that would appear more complicated than the early generations, and but for the following line of thought would require further explanation. I let it rest for the moment, and must only touch upon some points that are to me especially difficult

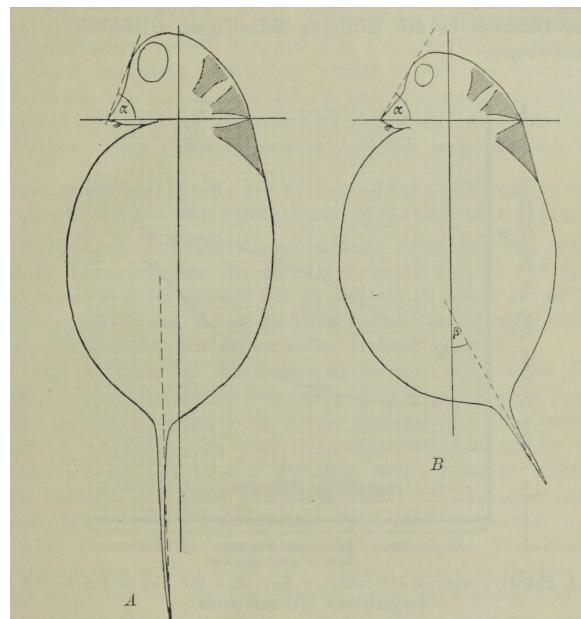


Fig. 13. Both elementary types of *Daphnia longispina* from the Obersee (A) and Untersee (B) of Lunz. (cf. the explanation of Fig. 2.)

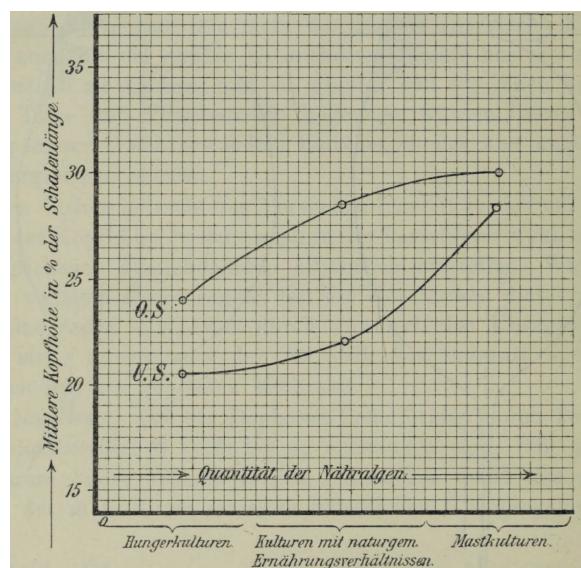


Fig. 14. Phenotype curves of the relative head-height for the two elementary types represented in Fig. 13.

and not quite settled. These regard the correlation between sexuality and head-height.

II. Analysis of the phenotypic and genotypic differences of the quantitative character of sexuality.

a. Exact determination and quantitative confirmation; causes of the differences We understand sexuality in this context as the tendency of an animal to have sexes ("strong sex") instead of being parthenogenetic ("degraded sex"). The sexual tendency expresses itself in the production of ♂♂ or of mature ♀♀ bearing ephippia (that is, fertilization-dependent) eggs. We will briefly examine and compare each instantiation of the sexual tendency. These stand in contrast to the asexual tendency, with its production of feminine subitan eggs. (The production of subitan eggs does not on its own suffice to determine sex; these eggs can still produce ♂♂.)

The sexuality of any given animal (♀) can be known best by examining its progeny, specifically by using the more or less developed formation of the latent eggs ("winter eggs") in the ovary as the observational criterion. For the most part it is sufficient to determine how many young ♂♂ and how many latent eggs of the total count to measure sexuality, and on the other hand how many young ♀♀ are derived by parthenogenesis. From this we obtain a specific numerical value measuring sexuality. For example, "sexuality" = 100% (i.e. only males and Ephippia) or = 50%, = 10% (i.e. half or 90% of the culture are parthenogenetically-derived ♀♀).

Therefore, the sexuality of our *Daphnia* is a measurable and quantitatively determinable singularity [????], just like head-height, spine length, egg production, movement and heart rate, phototaxis, etc. As we go on to learn [??konstatieren??], this character is very similar to e.g. head-height in the influence upon it of the relations of nourishment, so understandably we observe that the elementary types differ in a very analogous fashion in this matter also. Indeed, we find that sexuality differences offer our conclusions support just as well as helm development does in the preceding section. Sexuality differences of the biotypes are also continuously distributed and determined by the environment.

We can prosecute our analysis of this trait in the same way that we treated "helm-height", and thus we treat first the slightest natural differences of type and natural transitions, then the "artificial" transitions, and finally the behavior of different generations.

Up to this point we aimed to determine phenotype curves for each elementary type, and thus to itemize which sexuality degree each type demonstrates under each environmental condition (of nourishment, temperature, etc.). However, for now we will content ourselves with establishing that each elementary type has a distinct reaction norm for every character. In other words: the quantity of nourishment which must be given to a culture (of defined generation), such that for example 50% of the culture is parthenogenetic, is different for each genetically fixed landrace. We obtain a specific phenotype curve for each elementary type, that corresponds to the specific reaction norm of this character.

b. The beginnings of genotypical differences: natural transitions. When we search for the smallest heritable differences, and therefore towards the beginning of divergence, we also find forms for this character that do not differ at lower and higher degrees of some environmental parameter (with less and more nutrition), while they differ at middling supplies of nourishment. Such types show all possible natural transitions in their sexuality.

By way of comparison, one can at best pick out animals, belonging to middle generations of two biotypes, for which earlier and later generations often show a less clear reaction norm. (The early generations are in very many cases purely asexual, whereas the later are in great measure sexual.) With these middle generations one finds that one type with middle nutrition shows 50% sexuality, the other shows 75%, the third already is 100% sexual. All three biotypes are fully sexual when underfed, while with overfeeding all produce overwhelmingly parthenogenetic ♀♀. The plotted curves correspond completely to those given for helm-height (Fig. 12). They are merely somewhat complicated by the stronger generational differences, and we shall therefore not examine them more closely.

c. Artificial transitions, in agreement with natural environmental relationships. Likewise we find also for this character "divergences of the second grade" (S. 143) [determine where this falls in final version], which is to say elementary types, in the face of natural environmental relations hold themselves separate, but which can (for example) through overabundant nourishment make "artificial transitions".

I would like to bring up at least one example, representing an artificial transition between two extremely different cases. In some alpine pools near Lunz (at around 1600-1800m elevation) there lives a *Daphnia pulex (obtusa)*, which exhibits a sharply imprinted cycle. The animals emerge from the dauer eggs in approximately May-June, produce then great quantities of parthenogenetic ♀♀, which however as early as August-September go over to pure sexual reproduction. While the stimulus for this transition in the reduction of nutrition must be observed, it is likely that the overpopulation of the pond itself would cause this. In early fall then, there are only ephippia and dauer eggs observable, that now must lie 8 months long under ice and snow.

Now, I can treat just these *Daphnia* consistently with the heights of their assimilation [???] (rich food and middling to high temperature), and then hold them over a year long purely parthenogenetic, without observing in these cultures a single ♂ or ephippia. Through an unlucky mistake this culture was destroyed. My new cultures are 8 months old and show up until now a completely identical behavior. (The consequences of these observations for the now-so-often-discussed sexuality problem do not belong in this article; see appendix 4.)

Through my cultures I will make an artificial transition between the sexual relations of this high mountain form and the acyclical reproduction of many lake cladocerans, which again indicates that such differences, at least when

they are so large as in this case, cannot be derived by a discrete process. That they are environmentally determined is in this case plain; we can also as with head-height (S. 140 **find nums**) know, that they are similar environmental factors (chronically rich nutrition) that bring forth both acyclical colonies and our artificially acyclical cultures.

d. Reaction differences of generations, in agreement with the natural relations. Generational differences in sexuality are far more conspicuous but also rather more complicated than analogous differences in the reaction intensity of other quantitative characters, for example head-height (S. 143??). These differences in sexual tendency are exhibited even under similar life histories.

Different forms of the cyclically fluctuating sexual reaction norm (specific “generational successions”). If we take a large number of *Daphnia* local forms from very different situations and cultivate them from ephippia, taking care to maintain similar conditions of the environment, we observe a somewhat different generational progression of sexual behavior for each elementary type. We establish for each a distinct “generational succession” of sexuality for each.

However, we can immediately obtain a tally of categories, which nevertheless are connected by all possible transitions and intermediates.

- First category:

- (a) sexuality rapidly increases in the course of the initial generations (1-2-3) from 0% to 100% and is thereafter obligatory, which is to say that it solely dominates under all environmental relations from that point on.
- (b) The sexuality increases just as quickly, but it remains facultative and partial, which is to say that it can be satisfied with rich nutrition to produce some restricted parthenogenesis in varying degrees.

- Second category:

- (a) Sexuality gradually increases over the course of 10 or more generations from 0% to 100% and at last becomes obligatory.
- (b) The same, except that sexuality remains instead facultative and partial (in varying percentages).

- Third category:

1. Sexuality increases very nearly as quickly as the first category, but never quite reaching total dominance. The progeny of the persistent parthenogens (or the ♀♀ that can return to parthenogenesis) are almost completely asexual, but can regain sexuality gradually. After 6-12 generations these animals reach absolute and obligatory sexuality.
2. The same, except that the second period of sexuality remains facultative and partial.
- Fourth category: All generations are obligatorily parthenogenetic; which is to say that even under poor assimilation they will not fall into sexuality. (Also, we cannot let these cultures begin from ephippia or from dauer).

It would go far beyond our present purpose to study all of these categories in detail. What we wish to show, once again, is only this: sexuality is a quantitative character whose expression does not change discretely and suddenly. And second: this character's specific manifestations must be derived under the (direct or indirect) influence of the environmental relations.

Both of these conclusions proceed from the causative contingency [???ursaechlichen bedingtheit] displayed by this character, as the natural and artificial transitions bring forth. Both are illustrated by the different types of sexual generational successions, which surely derive their natural causes by these means.

we will now examine some typical cases from the above category IIa in Fig. 15, which prove to be approximately of the so-called “monocyclic” *Daphnia*. It concerns some races of *Hyalodaphnia cucullata* found by Borsdorf.

The shifting of the reaction-curves or phenotype-curves from one generation to follows here: In the first, generations obtained from dauer eggs, we find parthenogens at all levels of nutrition; the count of developed subitan eggs can indeed be forced nearly back to zero through hunger, however not without the development of ♂♂ and ephippia (dauer eggs) taking their place. Concerning this last reaction: after poor assimilation, sexuality appears in the following generations in ever-greater proportions, which is to say that it is a perpetually increasing nourishment that is necessary to maintain parthenogenesis in the later generations [??]. In the middle generations with middling nourishment we see sexuality of approximately 50%, and only observe pure parthenogenesis with rich nourishment. Later, an always higher environmental (assimilation) degree is necessary to maintain even partial parthenogenesis, and likewise we finally observe absolute (obligatory) sexuality at all levels of the environment, just as we could establish absolutely asexual behavior at earlier generations.

We bring ourselves yet further towards that “polycyclic” pool form, as we observed above (S. 146 **where??**). These *Daphnia* belong to the category 1a or 1b characterized above. Indeed, under my tutelage V. Scharffenberg has experimented at last upon a race of *Daphnia magna* (that also Kerherv and Issakowitsch examined) from ephippia. He found that the tendency to develop fertilizable dauer eggs emerges very quickly, already in the second and third generations. He established further that they arise also in the same ♀ as other eggs. In spite of this he could not fix obligatory sexual reproduction in the higher generations, as obtains for the Borsdorfer *Hyalodaphnia* and other Biotypes. V. Scharffenberg additionally made the interesting observation that a *Daphnia pulex* ♀ coming out of dauer stage is immediately able to produce a large quantity of parthenogenetic broods, can directly thereafter produce dauer eggs in response to the stimulus of poor assimilation. (This also most likely holds for his *D. magna* race). The production of ♀♀ by these *Daphnia*-types (after O. Kuttner's [Freiburg] and V. Scharffenberg's discovery¹⁵) is not influenced by level of nourish-

¹⁵Both works appear together in the “Intern. Revue der ges. Hydrobiologie” etc.

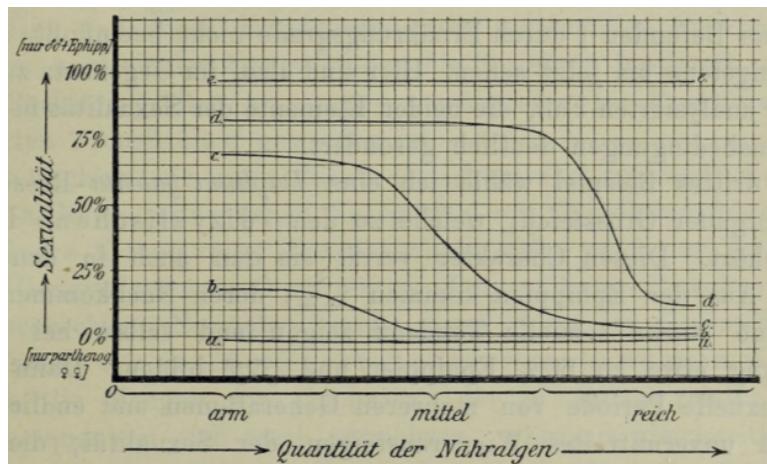


Fig. 15. Calculated phenotype-curves of sexuality for *Hyalodaphnia* (Borsdorf) for different generations. *a*, first generation; *b*, *c*, *d*, middling generations; *e*, later generations. Collected curves represented only for constant, middling temperature.

ment, at least so far as we can tell now. In contrast to the case that I have analyzed, these reports examine the two elements of sexuality in clear separation.

As a third example, I choose a *Daphnia galeata* race from the large pond of Torgau, to which the “dicyclic” *Daphnia* belong. These characters betray themselves only in cultures. From ephippia come ♀♀, whose descendants immediately show a strong sexual tendency and which themselves will show 60% ephippia and produce ♂♂, given good nourishment. After this follows an asexual period of multiple generations and finally a rather abrupt re-establishment of sexuality. The sexual animals develop by far into the majority finally, around 80-90% given good nourishment. Obligatory sexuality is not reached, as in the case of Fig. 15. In the course of further generations sexuality diminishes yet again.

These three examples serve as parerga [trans. note: Greek, signifying an embellishment] to the table given on S. 148 [update page] of the different categories of sexual generational progressions, which suffice well to demonstrate one thing that I desire to show. Namely, the specific quantitative characters of our *Daphnia* are endlessly complex quantities, whose genotypic bases (the heritably fixed reaction norm) are linked by manifold transitions from biotype to biotype, and impossible to consider as discrete from one another among the volatile particularities of environment and generation.

The other thing that I wish to show, which all the discussed observations should demonstrate, is the environmental dependence of all quantitative traits.

Relationships of the different types of specific “generational progressions” to the natural environmental relations. The periodic (and in certain regularities inherited) fluctuations of the sexual reaction norm can only be separated through the periodic fluctuations of precisely those agencies that induce this sexual reaction in their quantitative values.

Now, if we observe these periodic reaction fluctuations

arising in our cultures while the intensity of those agencies remain constant, then we can only adopt my opinion that through the longstanding and regular exposure of certain changing agent-quantities, certain changing reaction quantities are developed into a determined process through a fixation in the organism's reaction substrate **maybe fix this up a bit**¹⁶.

From this point of view we once again consider the categories of generational progression obtained from our cultures:

First category of generational progressions: Sexuality rapidly increases in the course of generations 1-3 from 0% to 100%: “polycyclic” denizens of small bodies of water. Normal causes of this form of generational progression is first the rapid depletion of the supplied food through the quantities of *Daphnia* rapidly reproducing in a relatively small amount of water, while this water simultaneously decreases rapidly from spring through summer due to evaporation and drying-out of the pond. At the same time, the accumulation of nitrogenous excreta (in the sense of Langhans, see his article in these proceedings) and the enrichment of the water with carbon dioxide through the *Daphnia*'s increased use of oxygen (soaring numbers of *Daphnia* and similarly decline of algae suspended in the water!) will work towards the curtailment of assimilation, this last being the actual causal stimulus for the increase of sexuality.

This cycle [??]: dauer eggs – 1 to 3 parthenogenetic generations – dauer eggs are the substrate (gonadal primordia and germ plasm) in which this reaction process plays itself out, forced firmly in this way in the course of very many generational cycles, such that today in many cases even without the habituated increase and decrease of the assimilation agents, if also mostly in moderated forms (facultative or partial sexuality also in the later generations, apart from the to-

¹⁶trans. note: Note again the clear idea of an evolved *genetically determined* reaction norm.

tal sexuality, which is then maintained openly) look over this one again??¹⁷.

Anyway, for now we cannot be certain which process has its course so fixed. Some examples might be: assimilated energy from such unusual doses of the input food; warmth etc. in the gonads; something like the expense of the previously assimilated cell materials, which remain "normal" and therefore carry out normal sequences of reaction; or whether (as is nearer to what we know) the unaccustomed magnitude of those agencies correspondingly changes, and thus abnormal stimuli are allowed to work upon the substrate (germ stock) [????]. Then this substrate would itself be the persistent locus of the sequences of reaction to normal, accustomed stimulus intensities.

In each case the heritable fixation of the specific generational progression establishes that we are in fact dealing with a genotypical character, and the obviously environmentally-dependent emergence of this character shows again clearly that these genotypical characters and their differences among our elementary types are environmentally dependent (and not mutations).¹⁸

At the same time, we wish to not neglect another possible cause that may materially play a part in the heritable fixation of the generational cycles, if it demonstrates its contribution under stringent and exact experimentation. This is the operation of natural selection (as rightly emphasized by Weismann), under which can be taken the surety of the eminently appropriate alternation between rapid parthenogenetic reproduction and the drought- and frost-resistant dauer eggs, because those individuals and populations that show this appropriate provision for less development are in point of fact threatened in their survival. Each freeze, famine, or drought of the water leads to the decided extinction of all of its resident Daphnia colonies that have not developed dauer eggs.

One need not attribute all the peculiarities of the organism to the action of selection if they can emerge by means of simpler or more direct means, but it would be nonetheless incorrect to refuse to consider natural selection when it so patently plays an enormous role in the phenomenon [*erscheinungskomplex*] that we busy ourselves about. The appearance of parthenogenesis—so useful for rapid proliferation—from the originally sexual reproduction is likewise attributable to selection, as the fine structural design [*durchbildung*] of the ephippia with their exquisite functionality.

In spite of this, we shall not at all concern ourselves here with the part played by selection in the emergence of Daphnia type characters. Selection consists of an indirect action of the environment. This, together with the direct environmental effects that we have so extensively discussed, constitutes the "environmental dependence" which we desire to demonstrate for the characters of our elementary types.

¹⁷Here again Woltereck anticipates genetic assimilation!

¹⁸Note that Woltereck argues not against *genotypes*, but against the *mutational origin* (in the sense of De Vries, not our modern sense) of these genotypes.

Second category of generational progressions: Sexuality increases gradually, in the course of numerous generations from 0% to 100%: these tend to be monocyclic Daphnia in larger bodies of water. The cycle ends either with obligatory sexuality or with rich nourishment parthenogenesis may persist in lesser or greater rates; that leads then to acyclic reproduction throughout.

Here the environmentally-dependent emergence of the progression is simpler and clearer to see than in the previous case, at least in the significance of its conclusion: the count of the parthenogenetic generations corresponds to the time of year, from spring—when the first ♀ hatches from the dauer eggs—to autumn—when assimilation is gradually reduced by the decline of both available food and temperature—through which the stimulus is given to increase sexuality. This regular onset (e.g. in the 10th-11th generation) of the assimilation decline appears to be so solidly impressed in the gonad primordium and the germ plasm that today we observe the accustomed sexual reaction emerge even without a seasonal diminution of the assimilation factors. If we talk about "qualities obtained by inheritance", we must be clear that such assimilation "qualities" (reaction intensities) and their alterations must concern not only the soma, but also at the same time the gonads and the germ plasm therein.

Compared to the end of the cycle, it is more difficult to understand how the beginning of the cycle corresponds to the environment of the monocyclic forms. In many colonies at least the first generations with obligate parthenogenesis experience less food and warmth than those generations later in summer. Nonetheless they are decidedly asexual at the beginning, and remain so with food shortages and cold, as V. Scharffenberg and I have shown with our cultures.

Other colonies begin their cycle first with richly available food and summery water temperatures. Have those early-appearing Daphnia experienced an alteration of the sexual reaction through a secondary adaptation, where their hatching from dauer eggs occurs earlier and earlier? Or maybe in the home waters of these colonies in March those "centrifuge plankton" develop richly that can pass through our finest nets, that Lohmann has discovered in the sea, and that I then found again in fresh water as the most important food for the Entomostraca? Or lastly, is that above-mentioned and still baffling correlation between lesser head-height and stronger parthenogenetic reproduction playing a role?

I will not discuss these possibilities here, or rather only indicate them, as they still await any exacting test. At present the observation suffices that the monocyclic reaction norm of sexuality must by and large have originate from the action of the environment.

Third category of generational progressions: Sexuality initially grows nearly as fast, even with rich food; the parthenogens continue to deliver reproducing ♀ descendants which are nearly all asexual but slowly become more sexual. These are the dicyclic Daphnia, which finish with higher rates of obligatory or facultative sexuality.

The causes of this singular rhythm of the reaction norm are not yet explained. Two potential causes are currently un-

der consideration. First possibility: through the dramatic reproduction in the first generation, available food is quickly used up in the original small home ponds of this type, and assimilation follows, and sexuality follows but remains partial, in order that for a minority, straitened parthenogenetic reproduction is preserved. Then, the numbers of the population decline precipitously, and the survivors find a newly enriched food supply (perhaps having to do with the manuring of the water with the innumerable carcasses of sexual animals), but increase again until the autumn, when assimilation drops again below category 2. Through this we see the emergence of the second slowly waxing sexual period.

Second possibility: The first sexual period of the dicyclic *Daphnia* is a reminiscence of the short summer, as it was after the last ice age, at which time our current *Daphnia* were in part derived, and which still acts to regulate these animals. The second sexual period is then a modern contrivance related to our current summer length. We do not need to discuss these two possibilities here (though the first seems more plausible to me), because in both cases it is the habit of the environment that underlies the constitution of the generational cycle. That alone is the important insight here.

Fourth category of generational progressions: Unconditional parthenogenesis under all environmental conditions, which above all allow egg development: these are the acyclic Cladocerans of the large lakes and maybe those waters which lie at warmer latitudes.

The environmental dependence of this extreme form of lifestyle lies plainly before our eyes. The acyclic races have lived for a very long time in waters that afford them the necessary materials all year long for sufficient assimilation, such that nearly facultative parthenogenesis (categories 1b, 2b or 3b, S. 148 **check page**) can be maintained, which then in the course of time solidifies into obligatory parthenogenesis.

The races that have developed this full sexlessness are the *Daphnia longispina-hyalina*, *cucullata*, and likewise the purely pelagic genus *Bosmina*.

Nonetheless, I bring up a colony of *Daphnia pulex* (which otherwise fosters polycyclic reproduction) from the warm lake of the Astroni crater in the Phlegraean fields. Since Spring 1906 this colony reproduced parthenogenetically without the appearance of a single ♂ or ephippium, when grown at 5° under meager food conditions. Instead they limit their egg production, and gradually cease egg production entirely and go extinct, but show no trace of sexuality. At the time of collection, I observed a very few ♂♂, so it is possible that this elementary type is just about to become fully asexual in the warm and food-rich Astroni lake. I immediately placed the animals in the hothouse of the Lunz station with abundant food; perhaps in my cultures this was merely the last prod to take in this direction. I come back to this briefly (S. 166 **seite 166 bitte**).

Therefore, we see that all the manifold and thoroughly “transgressive” forms of the *Daphnia* colonies lead back to the influence of the environment, as I believe they must. We can take from this phenomenon a second support that

is at least as powerful for our conception of the alteration of types, as we previously gained from the analysis of helm development.

III. Investigations on the appearance of regressive variation and experiments on its production and fixation.

These investigations involve two interesting characters of *Hyalodaphnia*: the nebenauge¹⁹ (accessory eye) and the “toothlet crowns”²⁰ of the young animals.

Both organs are normally missing from the local types that I investigated, and are rather originally endogenous features of the more distant cousins of the genus *Hyalodaphnia*, for example *Daphnia longispina-galeata*. Their recurrence in *Hyalodaphnia* consequently represents a regressive variant.

I studied these regressive variants (and others) from this point of view: can at least these characters of my *Hyalodaphnia*, the absences of nebenauge and the juvenile toothlet crowns, be discretely (e.g. mutationally) derived? Both characters appeared to me to be of a type with discrete characters without transitional forms and with no appreciable causal relation to any environmental dependence. **take another look at this**

a. The nebenauge of *Hyalodaphnia*.

The genus of *Hyalodaphnia* (from Schoedler 1866) is based on the absence of the nebenauge. However, in the intervening time Lilljeborg and others have acquainted us with a series of cases in which the nebenauge is also found in animals that show nearly all the characters of *Hyalodaphnia* (particularly the blunt rostrum with terminal scent antennae). Further, it was established that some lakes such *Hyalodaphnia* with nebenauge occur in spring, whereas in summer typical *Hyalodaphnia* are found. It is therefore already very probable that *Hyalodaphnia* without nebenauge might be able to have offspring with this pigment spot, and yield to them.

We can compile similar results from E. Wagler (Leipzig) and my own cultures (especially the Frederiksborger *Hyalodaphnia*) showing that such alterations certainly appear, and in both directions.

I could confirm experimentally that in earlier generations (after dauer) the nebenauge is more common and more completely developed than in the later generations.

Already this contradicts my original conjecture, that we would like the character to have to do with a sudden disappearance. The inherited primordium of this structure shows the same intermediating periodicity that we became acquainted with in the characters of head-height and sexuality: some generations show the ancestral behavior, and only some the new, negative character.

With further investigation it was shown that we are not really dealing with a discrete character (absence or presence

¹⁹trans. note: the German term appears to be accepted generally for this feature.

²⁰“Scheitelzaehnchen”

of a pigment spot), but rather all possible transitional forms between the original and the new behavior exist.

Indeed we find transitions in two senses: either the cells of the nebenauge are more or less maintained but the pigment is missing; or the pigment is in stronger or weaker development, but the cells are regressed. Each sign of the cell complex normally lying before the brain can be lost, and yet the characteristic black pigment granules are arranged, though only inside the brain matter (see Fig. 16, d).

The transitions concern interestingly not only the degree of development of these rudimentary organs, but rather also the level of heritability. I saw these pigment spots appear and then again disappear in different pure lines of the Frederiksborg *Hyalodaphnia* in a disordered, apparently capricious pattern. Then I observed however that in some cases ♀♀ had nebenauge, which inherited these regressive variants to a rather high degree.

With one such ♀ that I isolated based on the well-developed nebenauge of its mother and grandmother, I achieved a nebenauge heritability above 90%, which I also was able to select from the nebenauge-less *Hyalodaphnia* through only threefold selection and propagation of a *Daphnia hyalina* without nebenauge. In the ♀ with nebenauge, the regressive trait (the atavism) was so strongly impressed that some offspring with fully normal nebenauge (Fig 16, a) were achieved. On all accounts, the organ remained very variable thereafter, and some nebenaugeneless individuals appeared in most clutches, even under continuous selection.

I am attempting to influence nebenauge formation through the removal of light and through extreme temperatures, but for now have not obtained clean results from these experiments.

We are therefore dealing with a quantitative (and regressive) character, that indeed has no obvious relationship to the environmental conditions, but which through the aforementioned manifold grades of transitional forms can clearly only appear through a continuously distributed degeneration.

d. The toothlet crown of the *Hyalodaphnia*.

This inconspicuous organ is notable for my study and of high interest, because studying it has exposed that the helm primordial cells and the tooth crownlet primordial cells are one and the same in *Hyalodaphnia* and *Daphnia galeata*, and they are also found in many helmless races of *Daphnia longispina*. From this we can say with some certainty that the helm-building ability arises out of the older ability to generate the similar substrate of the chitinous toothlets. It is further found that transitions between typical “toothlets” and small superimposed head spikes.

It was further shown through the investigations of E. Wagler (which were carried out at the Zoological Institute at Leipzig under my direction) that with many *Daphnia* (*D. pulex*, *galeata*, *longispina*, and *Hyalodaphnia*) all possible transitional forms occur between the familiar “neck toothlet” characteristic of so many *Daphnia* (particularly ♂♂) and the typical crown toothlets of the young *D. galeata* and *Hyalodaphnia*. See Fig. 17 and 18.

We obviously do not wish here to further pursue a full

history of helm development²¹, but rather only to bring into focus the appearance of the toothlets as regressive variants.

Continuous emergence

While for many races of *Daphnia longispina-galeata* the appearance of a toothlet on the crown of a young animal is a normal realization of an expected character, for the races of *Hyalodaphnia* that I am familiar with this appearance is only observed in isolated cases, in which it is understood as a regressive variant.

For this character we also find immediately next the manifold transitional forms: 1. in degrees of development, 2. in prevalence of occurrence, 3. in heritability, 4. in different strong tendencies for different generations. We have also noted certain complete parallels to previously observed regressive characters.

1. The toothlet can be longer or shorter, even unto merely a slight thickening of the chitin (Wagler). Further, it can vanish without a trace following the first molt, or the second, or the third as well.
2. A smaller or larger percentage of the young animals (from 0-100%!) may be furnished with this toothlet.
3. Isolation of those young ♀♀ with particularly well-developed and persistent toothlets in general does not enhance this character in the next generation (in relation to degree of development or to frequency of the character). On the contrary, many such simply retained their toothlets nearly always, completely like any phenotypic character. Nonetheless, in some cases I could demonstrate an increased frequency and therefore a greater heritability of the regressive variant, though still to a small degree than with the analogous nebenauge trait. While there I could achieve a nearly constant appearance of the old character (above 90%) through only threefold selection, the crown tooth has not yet yielded a heritable number above 50%.
4. The repeatedly referenced *Hyalodaphnia* of the Frederiksborg castle lake, for which I thank the friendliness of Dr. Wesenberg-Lund, show a curious difference of this character across different generations. From the ephippia, which I collected in March 1908 (in Naples, where I had the ephippia-material shipped to me), thousands upon thousands of young *Hyalodaphnia* hatched. These nearly all possessed (around 95%) a well-developed crown toothlet, that again receded in later generations. I believed then that I was simply dealing with an atavistic trait that was retained by sexually conceived animals but not by parthenogens, as for the Nauplius larvae and the nebenauge of *Leptodora*²².

²¹I believe that once the neck and crown toothlets were set to serve in breaking the dauer membrane (as it becomes hard earlier than the chitinous shell of the young animal, and thereby made it possible to rip through the eggshell).-If this hypothesis is borne out, we may possibly have also an explanation for the uneven distribution of this character across the different generations. See S. 160 S. 160 bitte.

²²trans. note: unclear citation

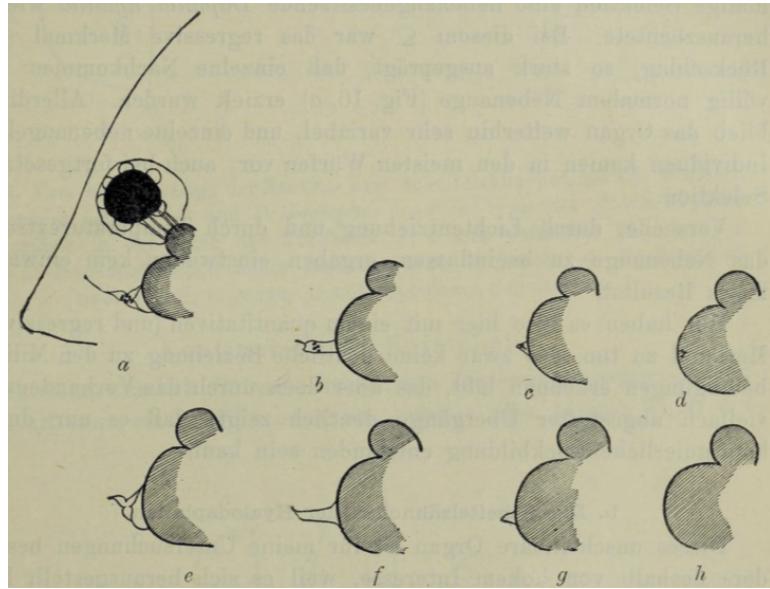


Fig. 16. Variations of the nebenauge in cultures of *Hyalodaphnia* (Frederiksborg). *a-d*, Pigment is contained, cells of the nebenauge in progressive deterioration; *e-g*, pigment is missing, the cells are however still present; *h*, cells and pigment grains missing.

However, this explanation was contradicted by the observation that from the ephippia collected in March 1909 from similar origin, young *Hyalodaphnia* were born that possessed that toothlet at only 15-20% frequency. There also, the grown descendants of these ♀♀ only partially thinned out for this trait, such that we can say at least that the tendency of this development in the first generation is as substantially heritable as in later generations; I can say it was indeed that it was similar to that calculated for the nebenauge above.

Influence of the environment

While there was no interpretable "environment-dependence" demonstrated for this pigment spot, an influence of the environment was apparent for the crown toothlet (also not yet understood in its causality).

Namely, I can artificially elicit parthenogenetically-derived ♀♀ with crown toothlets by transferring mature ♀♀ from higher temperature (25°C.) suddenly to a considerably lower temperature (12°C.), and leave them there to produce young. In order to obtain this result, the eggs must not yet be present in the brood chamber.

From this outcome, which I was able to obtain in repeated cases, I drew the preliminary conclusion that the conspicuous difference between the young *Hyalodaphnia* of 1908 and 1909 *ex ephippio* may be explained by this observation. The dauer eggs in winter 1907/1908 were exposed to a reduced temperature at some critical developmental phase, whereas the dauer eggs of the next year experienced no such condition.

If the connection between this environmental agency and its result of toothlet development is enigmatic for the moment, nonetheless we are confident that an environmental

dependence is at play in this character.

We can now place these investigated regressive characters together with the previously considered quantitative traits of helm-height and sexuality. Nebenauge and crown teeth also arise in all their known specific grades of development by a continuous path. These organs are reduced and lost continuously, just as we also see the possible degrees of development to be continuous, and are in certain cases heritably set. For one of the two characters there is additionally definite dependence on environmental changes; for the other one this is not yet proven, but it is also not certainly disclaimed.

IV. Attempts toward the elicitation of new genotypical quantitative characters through extended action of certain grades of environment.

Our observations so far chiefly concern the analysis of existing quantitative differences of elementary types. These differences were decomposed into their elements (the various phenotypes) in order that we might investigate their various causes. Then we could compare their overall differences (reaction norms), and draw some conclusions from their emergence.

Beside such analytical labors I have also busied myself about my Daphnia cultures, with the goal of heritably altering the reaction norms of my elementary types through longstanding exposure to certain grades of environment: in other words, bringing new genotypes into being.²³

It will astonish no one that in the short time of three years

²³Trans. note: see again the identity between "genotype" and "reaction norm" in Woltereck's conception.

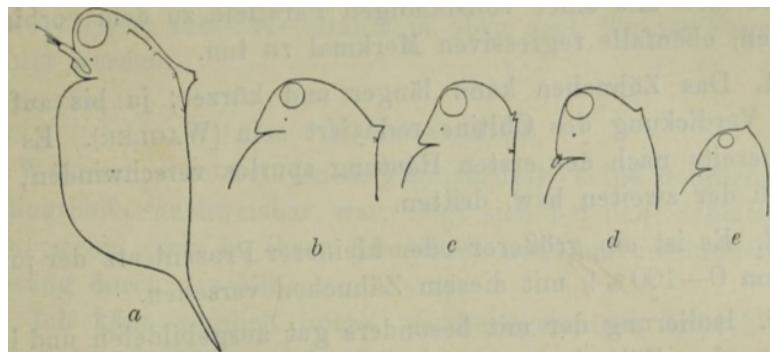


Fig. 17. Different locations of the toothlet between the neck and the crown after E. Wagler, for *Daphnia pulex* (a–b) and *Daphnia longispina* (c–e). a, typical ♂ *Daphnia pulex*, with neck tooth in the line of the head base; b, ♀ with simple toothlet; c, young ♀, neck toothlet pushed up higher; d, young ♂, neck tooth yet higher; e, young ♀ with typical crown tooth.

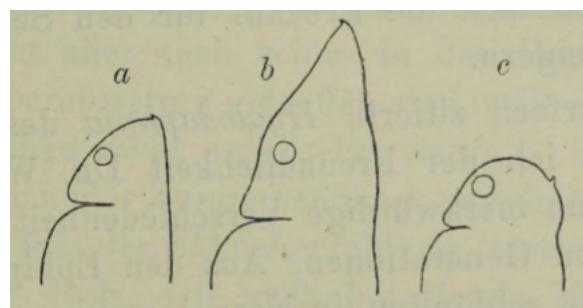


Fig. 18. Appearance of the crown toothlet for *Hyalodaphnia* (Frederiksborg). a, typical young form after hatching; b, the toothlet is retained in a fully developed ♀; c, the toothlet appears again for an old ♀ as a secondary growth between crown and back.

only incipient results were achieved; however these beginnings are encouraging, and they agree well with the conclusions of the analyses we have already examined. So consider these here as suggestions of similar work likely to be communicated very soon.

I restrict myself therein to considering the main points of a series of assays regarding a single elementary type: towards the investigations on the influence of extended hyperassimilation on the *Daphnia longispina* of the Lunz Untersee.

Emergence of a genotypically novel degree of head-height.

As I discovered in the work of previous years and have related above (**s.141 bitte**), the named type is accustomed to relatively poor nutrition and low temperatures, but through rich nourishment and higher temperature it becomes very rapidly changed phenotypically through an increased assimilation intensity. Moreover, through such extreme environmental changes these Untersee animals become very similar to Obersee *Daphnia* in some (not all) characters. These characters are: body size, head-height, spine length, spine angle, and brow angle (see Fig. 2). As already indicated, in this form they are not identical, and therefore the Obersee *Daphnia* is not merely a local modification.

Also, the assays that I describe now aim for the heritable fixation of one of these phenotypic changes (specifically

head-height), and surely will not lead us to confer upon the Untersee *Daphnia* the appearance of the Obersee type. The latter likely represents the original biotype, if we were to consider the appearance of regressive variants [????]. However, we can more or less arrange a phenotypically taller head for each elementary type of *Daphnia*; therefore I would have to predict that this same modification will behave itself in a decidedly regressive fashion.

The present exercise is much more concerned with bringing from the character of head-height a genotypically novel value, and thus, heritably altering the reaction norm. Should this be achieved, it must therein be shown that our elementary type also reacts to its ancestral habituated environment (in this case limited food) with an altered head-height, and therefore has actually become a new elementary type.

The arrangement of such an effort is rather simple: one takes great care to maintain constant increased nourishment and temperature, and also that the number of individuals per ccm of water does not change too greatly. Furthermore, one reins this in through continuous controls; for example, through isolation of some ♀♀ for fertilization. One then tests genotypical behavior from time to time (at a spacing of three to five generations), by isolating various animals into the original poor nutrition and determining the mean value of their offspring's head-height.

These tests revealed what I would like to share as a pre-

liminary result, and thus cautiously: there is a noticeable shift of the reaction norm in the direction of a genotypical fixation of the above-normal head-height that initially was evident in the phenotype.

This shift has revealed three periods as yet in its "history", in which the cultures behaved appreciably different.

The first culture period. This period concerns the first generations after the placement of some ♀♀ from the lake into culture glasses. In this new situation with rich *Chlorella* food and the high temperature of the hothouse, these animals straightaway take altered forms, which is particularly noticeable for the head-height. This first culture period is a time of conspicuous variability for our *Daphnia*, in particular with regard to the head profile. The rostrum appears truncated in some animals, and in others hooked or beaked, and in yet others appears with a notch either near to the eyes or near to the rostrum tip. The brow angle (Fig. 2a) is in these cases consistently increased; the reduced and receding head uniformly shown by *Daphnia* from the lake does not appear in these cultures.

However, the multitude of head forms turns out to be a very ephemeral and purely phenotypical phenomenon; when placed with sparse food similar to the natural relations of the lake, the animals mostly build themselves the typical Untersee head by the next molt. And, most interesting to us here: also the head-height changes to the original head-height in the time of a single molt after replacement in the old environment.

The second culture period. This period begins 3-4 months post captivity. It is characterized by development of a singular head-form with weakly concave brow contours. The aberrant profile forms appear more and more seldom, and similarly deformities of the eye, the spine and mantle, hermaphroditism, and lastly the above-mentioned spontaneous variants (S. 137 **seite 137 bitte**) become much rarer. All of these phenomena are characteristic of the beginning of the acclimatization, and also appear specifically when the cultures are contaminated by development of bacteria in addition to *Chlorella* or *Kirchneriella*, or through other influences. However, these same contaminants are less active in the second culture period, so one may readily speak of a continuous acclimatization to the environmental conditions (less room, rich food, high temperature). The *Daphnia* are now less delicate in their constitution, and similarly also less labile in their phenotype.

Most importantly, it appears that the animals do not resume the earlier form so quickly upon replacement in the original environmental circumstances (slim food, low temperature). Younger ♀♀ (only ♀♀ were considered) need more molts to reach the original head-height phenotype, and older ♀♀ overall cease to have entirely so small a head as the level of nutrition would seem to dictate. Nonetheless, the offspring born in the original environment of these same ♀♀ have the original mean value of head-height from the start.

A sharp division of this second culture period from the first and third is of course not possible; however I would like

to make a boundary between the behavior of the offspring of the third period mentioned below and the behavior of the older ♀♀ of the first period.

The third culture period. This period has finally just now (June 1909) been reached by some of my cultures (from late summer 1907). It shows itself as an important modification thus far, such that the offspring of high-headed females also show a greater mean value of head-height when they are raised with the original, straitened nourishment and with low temperature. Indeed they may not only be born in the original environment, but their mother females may have ovaries with young ovaries not yet divided into germ groups (egg + 3 feeder cells) when they are removed from rich food and placed with poor food. In other words, they first produce eggs in the original environment. Thereafter the hatched young animals had noticeably higher heads than we would expect from the original reaction norm of this elementary type for this degree of nourishment. In each case, an alteration of the reaction norm has occurred, or perhaps such a change is shown to us here at its first beginning. Thus we would be working with an alteration in the first generation raised under the old environmental conditions. The egg development leading to this generation occurred again under the original poor nutritional relations, so we deal with more than a mere "after-effect" of the rich nourishment. A full sharp division of the terms "after-effect" and "gradual type-altering environmental effect" is unfeasible.

Also, if one would prefer to interpret this reaction alteration as an "after-effect", it is nonetheless unmistakably a step forward from the merely phenotypic to the genotypic alteration of the head-height and still agrees well with the greater part of the analytical results shared in this article.

Genotypical alteration of sexuality. As we have just subjected the "sexuality" of *Daphnia* to a thorough investigation, so here I would like to briefly indicate that this character too appears to suitable for the study of the heritable influence of sustained environmental exposure. This is particularly true in such cases where there already exists a tendency in the animals towards labile sexuality in one or another direction.

A pronounced tendency towards asexuality (acyclism) plainly exists in many races of cladocerans, especially for pelagic *Daphnia* and *Bosmina*.

Both Lunzsee *Daphnia* (Untersee and Obersee varieties) are in an apparently advanced stage of development in this direction. This seems to agree with my observation that each of *D. longispina* cultures nearly completely loses the propensity fo sexuality when left in the warmhouse with rich food.

Moreover, in each case the later generations thus acclimated lose the ability to react (through sexuality) to minimal food and temperature.

Just so may the above-mentioned complete asexuality of my Astroni *Daphnia* (*D. pulex*) lead back to a modification through the action of the environment. See **Seite 155**.

And finally, the polycyclic Daphnia of the Alpine ponds appear to become sexual even when they are hindered by rich assimilation (**Seite 146**), driving off the parthenogenetic tendency as if they were transplanted to meager food and temperature. Here also the original reaction norm shows itself to be altered.

Clearly all of these findings require a test of many years. Nonetheless, it seems to indicate to me that I should bring these Cladocerans to the attention of my colleagues, as they are apparently discovered in a period of lability. The extraordinarily rich array of morphological and physiological characters gives witness to this lability.

Appendices

The causes of continuous (fluctuating) variation in Johannsen's work and my own. (from page 125.)

A crucial advantage of our materials (Daphnia) for our investigations is as follows: in each plant culture, as for example used by Johannsen, even under the most careful attempts to make experimental fields or patches like, there are **heritable?** differences in the environmental conditions of particular individuals. The same is true for fertilization and watering, the provision of room for growth, and further the available light for growing plants, and finally the quality of the soil for the sown seeds find for their rooting; all of that varies from individual to individual. And these are the principal causes for the individual, fluctuating variation, which is expressed in the frequency curve. The other causes rely on the quality of the sown grains, and thus (besides any differences in germ plasm) the mass of the nutritional material given to the embryo, in the thickness of the husk etc.

By contrast, with my Daphnia cultures the alikeness of the environmental conditions within the culture glass is absolute. If I fill such a glass with filtered water and load it with a dosed Chlorella quantum, the Daphnia living therein will experience completely alike conditions of existence. In there, they move unceasingly in the entire volume of water, and there is no difference from place to place. And there the suspended algae cells are filtered completely out of the water by the beat of their weir-like appendages, so the nutritional content of the water is like, and similarly the light and air concentrations and the water quality.

The small individual differences observed as yet are therefore entirely attributable to bodily differences which the animals have from their birth. They are therefore (barring differences of the heritable disposition) only differences in the nourishment of egg and embryo in the mother's body that cause fluctuating variation in the Daphnia of such cultures.

And so, for us the conditions of the experiments are clearer and simpler than for the botanists. They must reckon with two groups of individually varying and unknown quantities (embryo nourishment and individual position effects), and may never know how far their variants are attributable to one factor or the other. If they then go on to analyze the changing influence of the altered environment, they must hold always two different environmental influences separate: the general one that is "different throughout life history" (Jo-

hannsen, p. 219)²⁴, and the individual one inside of a "given life history", which is to say in one of the so-far-as-possible alike culture patches.

Logically, Johannsen distinguishes between two types of environment-dependent variability, the collective or "group variability" that follows from general environmental differences, and the fluctuating variability (in the narrower sense) that follows from specific (individual) environmental differences. This necessary complication obviously does not reflect actual (objective) differences, but rather only methodological differences: for a given individual, it matters little whether it is affected by a special or general difference in the environment. Only the degree of our understanding differs: in the first case we know nothing about how far the individual form may be blamed on the environment (and which factors specifically); only in the second case, where the entire culture is changed in some certain way, do we know which factors of the environment we may thank for the general form of the individuals. But also in this case there are many individuals that in spite of an enriched life history (e.g. manuring) have in their own case met adversity (e.g. through crowding). Through all this the factor of "environmental influence" becomes something vague and unreliable in these experiments. It therefore becomes understandable why when for example Johannsen dealt with it rather little in his fine experiments on selection in pure and mixed populations (and in these rare cases, never treated it numerically).

Appendix 2: Towards metabolic physiology of the cladocerans (from p. 127).

The volatility of the reactions of form paint an extraordinarily complicated but also especially appealing picture, demanding treatment with physiological methods. Unfortunately it has not been my luck to attract a physiological-chemical collaborator to this problem, who would obviously have to develop their own specific methods for these small creatures. In any case it would be necessary to have gratuitous quantities of Daphnia for each single phage of the assimilation process to conduct a comparative study, such that each stage unfolding from famine to the different grades of assimilation intensity can be observed without resorting to microchemistry. And if it should prove too difficult to attack conflicting assimilation processes individually, similarly, our understanding of overall metabolism in this organism is so poor that exacter investigation of the present phenomena concerning nutrition would be as welcome as respiration. There, we can follow my algae methods of feeding to make quantitative and qualitative analysis, taking also into account the metabolic byproducts in the water of the culture to make chemical processing possible. Of course, the solid, dissolved, and gaseous materials must be simultaneously investigated, for example because in excretory matter the oxygen and carbon dioxide in the water will be dependent upon one another. Dr. Langhans (see his submission to these proceedings) has presented fascinating work concerning how the accumulation of metabolic byproducts damages

²⁴trans. note: presumably *Elemente der exakten erblichkeitslehre* cited above.

the vitality of Daphnia. This often appears in my cultures, though very commonly there is no harm whatsoever, as in some cases when (“acclimated”) animals were kept living in a small tube in great numbers for over a year. I believe that differences in the water’s content of O and CO_2 sometimes yield a harmful effect of the metabolic byproducts, and sometimes not. *Daphnia magna* appear to be particularly resistant to accumulation of excretion materials in the form of O depletion and CO_2 excess, but only under such specific circumstances, which we do not yet know entirely. In the warm-house in Lunz I have repeatedly obtained cultures of *D. magna* in which not only *nearly* but *actually* developed a solid mass of ♀♀ Daphnia, such that the animals could not move freely; each was completely wedged in between the others. (This state of affairs occurred partially through reproduction, and partially because I allowed the water to evaporate.) There was in this water no trace of vegetation and the animals fed on stirred-up particles of bacteria-rich sludge that covered the bottom of the container. These cultures converted in part to sexual reproduction, though for weeks they remained in part weakly (1-2 eggs) parthenogenetic. I noticed no sign of degeneration. Where did these animals obtain the necessary oxygen (at water temperature $\approx 20^\circ!$) and how did they deal with carbon dioxide and excreta?? In general *D. magna* is rather sensitive to water deterioration. Freshly captured animals placed in great number in a glass tube often suddenly go extinct in hours, evidently due to O -starvation and CO_2 -poisoning; Knorrlich²⁵ and Langhans (as shared in friendly correspondence) have also repeatedly observed that such Daphnia suddenly die out during thunderstorms. The mass balance of “acclimated” animals must be completely altered. Hopefully the presentation of Langhans’ interesting researches on excreta will give us a physiological view regarding the question of metabolism. And hopefully through my correspondence and his, further collaborators (specifically chemical) can be found.

Appendix 3: Possible modes of formation of partial modifications of the reaction norm.

TRANSLATION IN PROGRESS

Acknowledgments

The digital version of the original article was digitized and hosted by the Harvard Biodiversity Heritage Library. I am grateful to AAAI and various people for implementing the AAAI style available on Overleaf used for this translation, and the www.dict.cc online dictionary as a general translation resource.

²⁵Ploener Forschungsberichte 1902