

Ephemeral pools as stressful and isolated habitats for the endemic aquatic resurrection plant *Chamaegigas intrepidus*

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with 4 figures and 1 table

Abstract. Ephemeral pools are widespread in (semi-)arid regions, where they form unique, mostly isolated habitats in an otherwise dry landscape matrix. They are characterized by extremely variable conditions, the most important stress factors being (1) frequent and fast desiccation and rehydration, (2) long dry seasons, (3) intensive solar irradiation and high temperatures during the dry season, (4) large diurnal oscillations of O₂ and CO₂ concentration and pH in the pool water, (5) extreme nutrient shortage, especially nitrogen. One functional plant type being highly adapted to these stressful conditions are aquatic resurrection plants, i.e. poikilohydric cormophytes like *Chamaegigas intrepidus* (Scrophulariaceae). This species grows endemically in ephemeral rock pools on granite outcrops in Central Namibia. *Ch. intrepidus* shows a number of anatomical, biochemical and physiological adaptations to the complex of extreme habitat conditions such as contractive xylem, velamen radicum, accumulation of abscisic acid, dehydrins and carbohydrates during desiccation. Nitrogen deficiency is partly avoided by acquisition of amino acids and an efficient utilisation of urea. *Chamaegigas* populations on single inselbergs are genetically very highly isolated, whereas gene flow between sub-populations from different pools on one inselberg is rather high. In accordance with the predominantly outcrossing breeding behaviour, most genetic diversity is found within a pool. A habitat model showed clear niche partitioning between *Ch. intrepidus* and the less desiccation-tolerant species *Limosella grandiflora* with respect to depth of the pools. The specific constitutive dehydration tolerance mechanisms displayed by *Ch. intrepidus* are discussed as a vital adaptation to the low predictability of the occurrence and duration of periods favourable for growth and reproduction.

Keywords: poikilohydric cormophyte, desiccation, niche partitioning, gene flow, inselbergs, Namibia.

1. Introduction

Ephemeral pools drying out at least during part of the growing season are found world-wide, being especially widespread in arid and semi-arid regions (WILLIAMS 1985). Thus, due to their aquatic nature, they form unique, mostly isolated habitats in an otherwise dry landscape matrix. As temporarily water-filled depressions on a variety of geological substrates, they

offer particular environmental conditions for plant growth, animal life and microbial activities. Their most striking character are the extremely variable conditions both on a diurnal and seasonal time scale (SCHOLNICK 1994). The period of desiccation can last from a few days to several years, dependent on precipitation patterns and evaporative demand of the atmosphere (WILLIAMS 1985). Concomitant to the fluctuating water status, these habitats are characterized by large oscillations of temperature, O₂ and CO₂ concentration, pH and chemical composition of the water (SCHOLNICK 1994). Moreover, both irradiation and temperature can reach levels causing damage to single cells or death of the whole organisms. Consequently, temporary pools are stressful habitats not only in the dry state, but also when being water-filled.

Ephemeral pools are often found on flat inselbergs, either on top or on gentle slopes (PÖREMSKI & BARTHLOTT 2000a). The monolithic rock outcrops abruptly rise from the surrounding plains, from one to several being grouped together. A specific inselberg may harbour more than 100 pools (KRIEGER et al. 2000). In most cases the individual pools lack any direct aquatic continuity, except perhaps for a short time when being flooded. Therefore, for the most part they represent both hydrologically and biologically isolated systems. This means that even in pools close to each other limnological characteristics can be rather different, affecting structure and dynamics of the biocoenoses. Moreover, gene flow due to either exchange of organisms or dispersal of resistant propagules (eggs, spores, seeds, pollen etc.) may be highly restricted at least for inselbergs being isolated by a matrix with low connectivity (e.g. large savannas unsuitable for pollinating insects).

As a consequence of the high variability and therefore low predictability of environmental conditions many ephemeral pools are inhabited by either cryptogames or a low number of ephemeral macrophytes. According to WILLIAMS (1985), short-lived, unpredictably filled pools do not harbour truly aquatic macrophytes, but may support flooded terrestrial vegetation, whereas in pools which are predictably water-filled for several months annual aquatic macrophytes will grow. On a large number of seasonal pools on inselbergs in the Ivory Coast KRIEGER et al. (2000) found therophytes with 24 out of 33 species to be the dominating life form. These authors ascribed the low proportion of aquatic species to the high environmental stochasticity, especially low reliability of water, for these West-African compared e.g. to Zimbabwean inselbergs on which aquatic members of the Scrophulariaceae dominate (e.g. *Lindernia spec.*). Similar to Zimbabwe, ephemeral rock pools in Namibia are characterized by the dominance of two aquatic members of the Scrophulariaceae, *Limosella grandiflora* and *Chamaegigas intrepidus*. Furthermore, in deeper, mostly water-filled pools *Aponogeton desertorum* (Aponogetonaceae) may be found (GAFF & GIESS 1986).

Both *Ch. intrepidus* and *L. grandiflora* are so-called resurrection plants, i.e. poikilohydric angiosperms. These plants survive intensive dehydration with a water loss of up to 80 to 95 % of their water content at full turgor

and subsequent rehydration (HARTUNG et al. 1998). Resurrection plants occur on all continents except Antarctica, but are concentrated in the Southern hemisphere, i.e. southern Africa, eastern South America and western Australia (ALPERT 2000). The phenomenon of desiccation tolerance has evolved in eight angiosperm families only (GAFF 1989). Most of the poikilohydric angiosperms are found within the Velloziaceae (> 200 species), others in Cyperaceae and Poaceae (POREMBSKI & BARTHLOTT 2000b). Among dicotyledonous species, most resurrection plants are found within the Scrophulariaceae. According to FISCHER (1992), nine of the ten African *Craterostigma* species and all the 15 African *Lindernia* species are poikilohydric. In contrast to cormophytes, desiccation tolerance is widespread in thallophytes (algae, lichens, fungi and mosses). HARTUNG et al. (1998) hypothesized that the reason for the low frequency of desiccation tolerance in vascular plant bodies (apart from low sensitivity to dehydration in seeds or pollen) is the high metabolic cost which is detrimental to competitive strength or reproductive performance under most growing conditions.

This paper reviews the mechanisms of adaptation to complex stressful environmental conditions in ephemeral pools on isolated granite outcrops in Central Namibia which are the only habitat of the resurrection plant *Chamaeigigas intrepidus* endemic to this region. First, after a short description of the species' morphological and biogeographical traits, limnological features with special attention to those factors causing abiotic stress are considered along with the plant's physiological mechanisms of resistance. Second, the effect of geographic isolation on gene flow and the genetic structure of *Chamaeigigas* populations at the different levels of its hierarchic distribution patterns will be shown. Third, aspects of niche partitioning between *Ch. intrepidus* and *L. grandiflora* in different pools will be discussed. This review concludes with implications of life history parameters in view of the extremely stochastic, unreliable nature of environmental conditions in these stressful and isolated ephemeral pools.

2. Habitus, phylogeny, distribution and habitat of *Chamaeigigas intrepidus*

Ch. intrepidus Dinter (formerly *Lindernia intrepidus* Obermeyer) is a tiny aquatic plant with two types of leaves: 8 to 15 mm long lanceolate submerged basal leaves on a short main axis and two decussate pairs of sessile floating leaves on top of a thin, 1.5 to 10 cm long stem. Within the centre of the floating rosette, two flowers are produced (HEIL 1924). Whereas submerged leaves are pale green at the distal and white at the basal part, floating leaves are dark green with purple margins and purple abaxial surfaces. The flower corolla is white/pink. The bilabiate appearance is dominated by the larger (6 to 7 mm long, 5 mm wide) lower central lobe, whereas the lateral lobes in the upper lips spread outwards only 4 mm (long) by 3 mm (wide). Due to the slightly protandrous character of the flower, self-deposition of the granuliform, light-yellow pollen is possible.

The flowers do not produce nectar, but are characterized by a distinct scent. Seeds are minute, about 1.5 mm long and very rugose (SMOOK 1969).

Ch. intrepidus is a member of the tribe Lindernieae (Scrophulariaceae s.l.), which has approximately 170 species in Africa (FISCHER 1992). The plant is closely related to species from *Lindernia* sect. *Lindernia* and sect. *Linearifoliae*. It occurs endemically in Namibia at the south-western corner of Lindernieae in Africa (Fig. 1).

Within its range of distribution, *Ch. intrepidus* grows exclusively in areas with granite outcrops in the semi-desert and savanna transition zone (GIESS 1969, 1997). The habitats of the species lie in the semi-arid region with 160 to 570 mm annual precipitation, with rainfall on only 20 to 70 days during summer (November to April), and a high variability from year to year (HICKEL 1967). Thus at the farm Otjua (Omaruru District, Namibia,

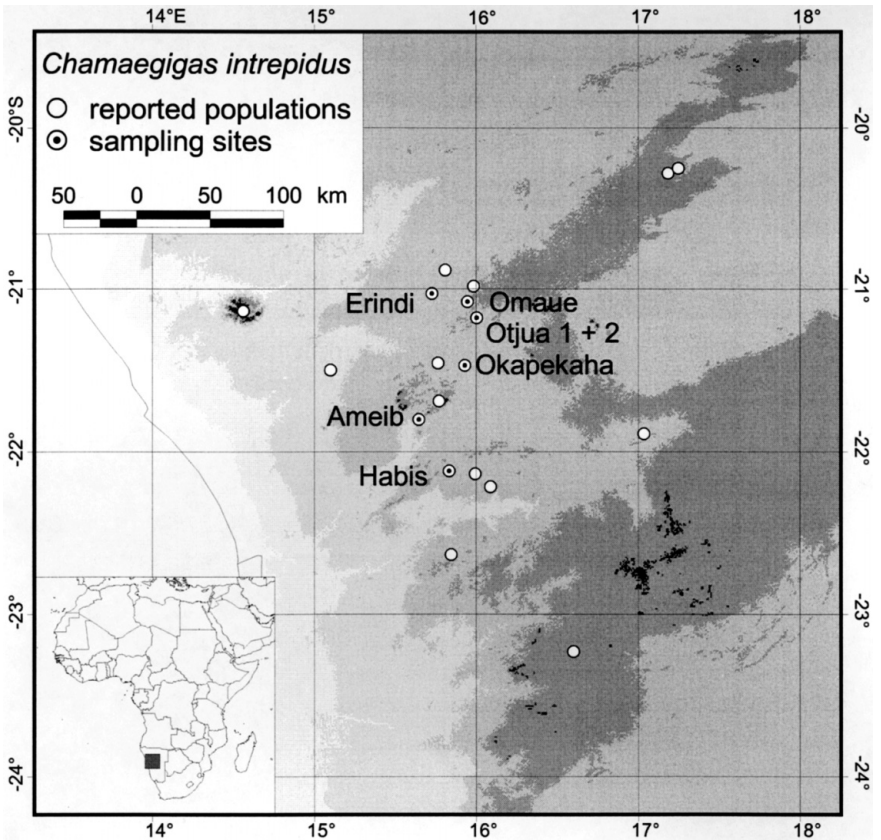


Fig. 1. Reported locations (FISCHER 1992, GIESS 1969, SMOOK 1969, CRAVEN & CRAVEN 2000, JÜRGENS & BURKE 2000) and sampling sites included in the current analysis of *Chamaegigas intrepidus*.

21°10'S, 16°E), where most of the studies described in this contribution were performed, precipitation in the season 1996/97 was much higher (453 mm) than in the drier seasons 1995/96 (179 mm) and 1994/95 (237 mm).

Ch. intrepidus grows in shallow pools (maximum depth 15 to 20 cm) which usually dry out completely during a sequence of several rainless days when average daily evaporation rate amounts to 12 to 25 mm (HEILMEIER & HARTUNG 2001). These pools may either occur isolated on the slopes of the rock outcrops (Fig. 2a) or clumped on flat tops of the outcrops (Fig. 2b). During periods of high and frequent precipitation (20 to 30 mm per day on several consecutive days) water from overflowing pools may connect adjacent pools. The area of the pools ranges from 0.4 to 96 m² (median 2.7 m², n = 96). *Chamaegigas* plants may either grow dispersed or in one to several dense patches (median size: 0.018 m²) per pool. The bottom of the pools is covered by a thin layer of mineral (quartz sand) and/or organic debris from decomposing algae and daphnias, animal faeces and leaf litter.

3. Abiotic stress factors and anatomic and physiological adaptations of *Ch. intrepidus*

3.1. Desiccation

The most prominent environmental feature of the species' habitat is the extreme drought stress caused (i) by a long dry season and (ii) frequent desiccation events during the wet season, when only a few (5 to 12) rainy days alternate with a number (up to 60) of dry days (HICKEL 1967). Over the whole wet season, pools may be filled with water for some 40 to 85 days only. Consequently, during a single rainy season *Chamaegigas* plants may experience 15 to 20 rehydration-dehydration cycles (GAFF & GIESS 1986). *Ch. intrepidus* shows a number of adaptations to these stochastically fluctuating hydrological conditions both on the anatomic-morphological and the molecular-biochemical and physiological level.

3.1.1. Anatomical adaptations to desiccation stress

Hydrated resurrection plants generally do not exhibit xeromorphic anatomical features, and *Ch. intrepidus* in particular does not possess any well-developed traits to protect it from water loss like a thick cuticle impermeable to water. Among all poikilohydric cormophytes, *Ch. intrepidus* shows the fastest rate of water loss, being air-dry within one hour (GAFF 1977). The fast rate of dehydration can only be tolerated by anatomical peculiarities at different levels from the cell to the whole organ. The length of desiccated submerged *Chamaegigas* leaves is only 10 to 20% of the hydrated leaves (HEIL 1924, HICKEL 1967). This drastic shrinkage is only possible by the unique existence of contractive tracheids (SCHILLER et al. 1999). The



Fig. 2. Ephemeral rock pools on granite outcrops on the farm Otjua (Omaruru District, Namibia). – Fig. 2a: The grey floating leaves and the flowers in the centre of the single pool are from *Limosella grandiflora* plants growing in the deeper part, whereas *Chamae-gigas intrepidus* (dark floating leaves and flowerheads at the fringe of the pool) grows in the shallow zones. Fig. 2b: Cluster of pools on the flat top of an outcrop. Note the varying cover of the pools with *Ch. intrepidus*. Black rims around the pools originate from cyanobacteria and indicate maximum water level, black stripes of cyanobacteria between individual pools indicate pathways of water when pools are overflowing.

shrinking process is accompanied by intensive wrinkling of the epidermis, which is caused by a tight connection between the plasma membrane and the cell wall resulting in contraction of the whole cell during dehydration. Through this cytorrhysis plasmodesmatal connections between cells are maintained (HARTUNG et al. 1998). During rehydration, water uptake may be promoted in the folded leaves by capillary trapping of water in leaf invaginations (KLUGE & BRULFERT 2000).

In contrast to submerged leaves, neither floating leaves nor roots show such a dramatic longitudinal shrinking upon dehydration. Roots, however, drastically shrink in the lateral direction by 30 to 35 % due to an exclusive shrinkage of the rhizodermis and the exodermis (HEILMEIER et al. 2002). The large single-layered cortex cells, however, do not collapse, not even shrink after complete desiccation, although they have extremely thin walls. Due to their utmost stability cortex cells prevent roots from shrinking. Thus maintenance of the physical root structure stabilises the sediment of the pools during the long dry winter season (HEILMEIER & HARTUNG 2001). In the dehydrated state, the shrunken rhizodermis and exodermis cells form a velamen radicum (HEILMEIER et al. 2002). Equivalent structures have been found in adventitious roots of tree-like Cyperaceae and Vellociaceae, where they are assumed to allow for rapid absorption of water during rehydration (BIEDINGER et al. 2000). On the other hand, water loss by roots may be slowed down by pads on the exterior walls of short cells within the dimorphic exodermis, a trait which has not been found in other aquatic plants until now (HEILMEIER et al. 2002).

3.1.2. Adaptation to desiccation stress at the molecular, biochemical and physiological level

For resurrection plants, a whole plethora of ecophysiological adaptations to desiccation from gene expression and regulation to whole plant metabolism have been described (see e.g. recent reviews by HARTUNG et al. 1998, KLUGE & BRULFERT 2000, SCOTT 2000, ALPERT & OLIVER 2002, BUITINK et al. 2002, Proctor & Pence 2002). According to KLUGE & BRULFERT (2000), for desiccation tolerance two basic strategies are realised: (i) limitation of protoplasmatic damage by synthesis of protection compounds, (ii) repair mechanisms. As discussed in HEILMEIER & HARTUNG (2001), protection of cellular integrity is considered to represent the major component of desiccation tolerance in *Ch. intrepidus*.

Although the exact mechanism of perception of water loss is still not clear, the decisive role of abscisic acid (ABA) in the signal transduction path has been clearly demonstrated (HARTUNG et al. 1998). In *Ch. intrepidus*, ABA concentration increases 20- to 30-fold during dehydration in submerged leaves and roots, whereas in floating leaves the increase is always less than fivefold (SCHILLER et al. 1997). The increase in ABA concentration is correlated with the decrease in osmotic potential, without any threshold, which indicates a rather sensitive response of ABA biosynthesis to dehy-

dration. Due to a slow degradation rate, *Ch. intrepidus* maintains high ABA concentrations in hydrated tissues (HEILMEIER & HARTUNG 2001).

One stress physiological function of ABA in *Ch. intrepidus* seems to be triggering the formation of dehydrins during desiccation (SCHILLER et al. 1997). These proteins are thought to sequester ions to prevent damage to proteins and other chemical cell constituents by excessive salt concentrations due to water loss (ALPERT & OLIVER 2002). Another class of protective compounds are compatible solutes like non-reducing sugars. In *Chamaeigigas* leaves, especially stachyose and sucrose increase during dehydration (HEILMEIER & HARTUNG 2001). The protective role of sugars is seen in (i) the maintenance of hydrophilic interactions in membranes and proteins, (ii) vitrification, i.e. formation of a biological glass, which prevents crystallisation of the cytoplasm (BUIJTINK et al. 2002).

Maintenance of subcellular structures like macromolecules and membranes is vital for functional integrity of cells. A major indication thereof is photosynthesis. By measuring the diurnal course of O₂ concentration in the pool water, HEILMEIER & HARTUNG (2001) could show that *Ch. intrepidus* performs photosynthesis with submerged leaves even when the medium is completely devoid of CO₂, possibly by utilising HCO₃⁻. Using advanced techniques of chlorophyll fluorescence, WOITKE et al. (2004) could demonstrate a significant role of the floating leaves for total plant carbon gain. On the other hand, both desiccation tolerance and regeneration capacity after dehydration were not different for both leaf types with respect to photosynthesis (WOITKE et al. 2004).

3.2. High irradiance

Due to the exposed nature and the elevation (ca. 1500 m a.s.l.) of the rock outcrops, photosynthetic photon flux density (PPFD) regularly exceeds 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during summer (HEILMEIER & HARTUNG 2001) and even basal submerged leaves which are covered by a dense layer of floating leaves may experience PPFD > 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (WOITKE et al. 2004). Especially when the pools dry out, basal leaves are exposed to full sun. A major mechanism of these leaves to protect against high irradiance load is the folding and curling during the shrinking process described above (3.1.1.). Curling of older exterior leaves over interior buds may protect dried young tissue from photodamage (PROCTOR & PENCE 2002). Furthermore, accumulation of high amounts of anthocyanins causing the red colour of the floating leaves protects from the high UV radiation (FARRANT 2000).

3.3. High temperature

Already the German botanist K. DINTER who discovered the "Unerschrockener Zwergriese" ("undaunted dwarf giant", *Chamaeigigas intrepidus*) in 1909 near Okahandja in Central Namibia was very much impressed by the extreme habitat conditions, especially the high temperature and associated

low relative humidity of the air (DINTER 1918). Maximum temperatures of the rock surface readily exceeds 50 °C, with a diurnal amplitude of 30 °C (HEILMEIER & HARTUNG 2001). During days without rainfall, minimum air humidity decreases to 5 to 10%. Atmospheric temperature conditions are only weakly attenuated in the pool water, with maximum temperature reaching 35 °C during the day and minimum temperature at night being comparable to nocturnal air temperature. The high water temperature in combination with the high evaporative demand of the atmosphere causes evaporation rates of the pool water to exceed 10 mm per day vastly (HEILMEIER & HARTUNG 2001).

Especially when pools are not filled with water the drying *Chamaegigas* plants are exposed to temperatures which are some 20 °C higher than water temperature. Heat shock proteins which are structurally and functionally related to the dehydrins described above (3.1.2.) might protect the plant from heat damage (KLUGE & BRULFERT 2000).

3.4. Diurnal oscillations of pool water pH and CO₂ concentration

Large diurnal fluctuations in water temperature in combination with photosynthetic activity of the plants are the cause of diurnal oscillations of dissolved CO₂, with a complete loss of CO₂ from the water during daytime at temperatures above 30 °C and maximum nocturnal CO₂ accumulation due to respiration up to 0.4 mol m⁻³ (HEILMEIER & HARTUNG 2001). Similar diurnal oscillations of free CO₂ concentration have been found in seasonal pools in California, USA (KEELEY 1999). Aquatic plants may respond to a limitation of photosynthesis due to low daytime CO₂ concentrations by either Crassulacean Acid Metabolism (CAM) or C₄ fixation. As is the case with the C₄ plant *Orcuttia californica* (KEELEY 1999) aquatic leaves of *Ch. intrepidus* had no Kranz anatomy, but also floating *Chamaegigas* leaves lacked this distinct feature, in contrast to terrestrial *Orcuttia* leaves. Furthermore, CAM is not known for close relatives of *Ch. intrepidus*, and KEELEY (1999) found no CAM species with floating leaves in the Californian pools. Therefore it is concluded that *Ch. intrepidus* depends on C₃ photosynthesis.

A further consequence of the diurnal fluctuations of pool water CO₂ concentrations are large oscillations in H⁺ concentrations between pH 6.9 (median) in the morning and pH 9.3 in the evening. Maximum oscillations may be even larger (pH 5.8 to pH 12.0, SCHILLER et al. 1997). In spite of these dramatic external pH fluctuations, *Ch. intrepidus* maintains a rather stable intracellular pH (SCHILLER et al. 1998a).

3.5. Nitrogen deficiency

Generally, vegetation of rock outcrops is limited by the availability of mineral nutrients (DÖRRSTOCK et al. 1996). As a consequence, a characteristic functional type of inselberg flora are carnivorous plants, especially in ephemeral and permanent wet flushes (KLUGE & BRULFERT 2000). Similarly, in the pool water of *Chamaegigas* habitats, apart from major nutrients such as potassium, calcium, magnesium, sulphate and phosphate, the concentration of nitrogenous compounds like nitrate and ammonium is rather low (HEILMEIER & HARTUNG 2001). On the other hand, organic N compounds like amino acids and especially urea are more abundant. In accordance with the high supply of organic N *Ch. intrepidus* is able to take up amino acids like glycine and serine (SCHILLER et al. 1998b). The acquisition of amino acids may be especially important when, due to high pH values, ammonium is nearly absent from the pool water. The major source of NH_4^+ is urea, which can be utilised by *Ch. intrepidus* only after enzymatic degradation. The enzyme urease is very resistant against the harsh conditions, surviving temperatures up to 60 °C and high UV radiation during more than six months of dry season (HEILMEIER et al. 2000). This may be important for providing ammonium for plants resuming growth during the early part of the wet season when urea deposited on the rock surface by wild and domestic animals is flushed into the pools by the first rainfalls (GAFF & GIESS 1986).

4. Genetic diversity in *Chamaegigas intrepidus*

Endemic species like *Ch. intrepidus* show a reduced genetic variability relative to widespread species (HAMRICK et al. 1979). Furthermore, rare species restricted to small isolated habitats may become genetically isolated and develop pronounced spatial genetic structures. This genetic structure of populations results from processes leading to genetic differentiation (genetic drift and selection) and processes that homogenise gene pools (gene flow). The high degree of spatial isolation of *Chamaegigas* habitats (average distance between individual inselbergs ca. 25 km, cf. Fig. 1) was supposed to reduce gene flow among populations. For that purpose, genetic diversity within populations, and genetic differentiation and gene flow among populations were analysed by means of highly polymorphic AFLP (amplified fragment length polymorphism) markers (DURKA et al. 2004). A preliminary analysis of 71 *Chamaegigas* plants from 17 pools at seven sites (cf. Fig. 1) with three primer combinations was performed with hierarchical structuring of the populations (individuals nested within pools, pools nested within sites).

Genetic diversity among individuals seems to be high at the pool and site level, with no correlation between genetic diversity and population size (DURKA et al. 2004). Genetic differentiation between sites (17% of total variation) is higher than genetic variation among pools within sites (6%) as revealed by analysis of molecular variance. However, most variability

is present among individuals within pools (77% of total variation). Thus *Chamaeigigas* on the different inselbergs is highly differentiated genetically. Genetic differentiation is not correlated with geographic distance. Assumption of an island model and isolation by distance yields the average effective number of migrants per generation among sites to be 1.2, among pools 3.0. These spatial patterns of genetic diversity are in accordance with the breeding system and dispersal mode. Pollination experiments indicate that *Ch. intrepidus* is a predominantly outcrossing species. We found a great number of wild bees (*Liotrigona bottegoi*) and especially beetles (*Coandylops erongoensis*, family Malachidae) visiting *Chamaeigigas* flowers (DURKA et al. 2004). These insects will predominantly pollinate plants from the same inselberg, but are not restricted to single pools, leading to only slight differentiation among pools. However, only rarely will pollinators be able to migrate between different inselbergs. The tiny seeds that are well dispersed by wind will also predominantly accomplish gene flow among pools within the same site (NATHAN & MULLER-LANDAU 2000).

In conclusion, even small populations of *Ch. intrepidus* do not seem to be genetically impoverished. However, gene flow between isolated sites within an uninhabitable matrix is too low to counteract differentiation.

5. Niche partitioning

Since the complex stress factors described above act on a very short time scale with most *Chamaeigigas* plants being hydrated for three to five days only, *Ch. intrepidus* can survive only by the existence of constitutive mechanisms for desiccation tolerance (HEILMEIER & HARTUNG 2001). This is in contrast to other vascular poikilohydric species in which tolerance mechanisms are inducible by dehydration (PROCTOR & PENCE 2002). According to the "productivity trade-off hypothesis" (ALPERT 2000) this ability to tolerate desiccation entails costs that constrain growth and reproduction. On the other hand, the short period of only a few hours to days of photosynthetic activity when the leaf tissues are sufficiently hydrated reduces carbon gain significantly (WOITKE et al. 2004). Thus, due to its low competitive strength *Ch. intrepidus* should be restricted to habitats with most extreme constellation of ecological factors within the temporary pools. Already GAFF & GIESS (1986) pointed out that *Ch. intrepidus* occurs in the shallowest ephemeral pools, whereas *Limosella grandiflora* grows in deeper pools. In contrast to *Ch. intrepidus*, leaves of *L. grandiflora* are not desiccation tolerant, only the rhizomes survive dehydration up to 0% RH. Therefore, we hypothesized that within the pools investigated by us there is a clear differentiation between *Ch. intrepidus* on the one hand and *L. grandiflora* on the other with respect to maximum water level. This hypothesis was tested in a habitat model (KLEYER et al. 1999/2000) by establishing statistical relationships between presence or absence of the two species and site factors such as maximum water level in the pools (indicating duration of plants being hydrated versus frequency and duration of plants being dehydrated), depth of sediment (nutrient pool, long-term persistence of

plants by anchoring roots and rhizomes), type of sediment (organic debris versus mineral particles) and conductivity (nutrient concentration of pool water). Probability of presence or absence of species as dependent on site factors selected was estimated by logistic regression (FLOYD 2001) using SYSTAT 9 (SPSS 1999).

Confirming our hypothesis, maximum water level was a significant predictor for the presence of both species (Table 1). Probability of occurrence strongly declined with increasing water level for *Ch. intrepidus* (Fig. 3a), whereas it increased nearly exponentially for *L. grandiflora* (Fig. 3b). On an outcrop on the farm Otjua harbouring 63 pools 36 of which were populated by *Ch. intrepidus* no *Chamaegigas* plants were found in pools deeper than 12 cm, but most of the shallower pools were covered by this species to varying extent (Fig. 4). Among the other site factors tested none was significant for *L. grandiflora*, whereas for *Ch. intrepidus* both type and depth of sediment were significant predictors, but not conductivity (Table 1). Probability of occurrence increased for *Ch. intrepidus* with increasing proportion of minerals in the sediment (Fig. 3c), indicating that a high concentration of organic substances in pools with abundant plant and animal debris is detrimental to this species. On the other hand, a rather shallow layer of sediment (< 1 cm) already provides enough mechanical stability for the persistence of *Chamaegigas* (Fig. 3d).

Thus the contrasting desiccation tolerance of the two Scrophulariaceae species *L. grandiflora* and *Ch. intrepidus* (especially their leaves) is clearly related to the duration of pools being water filled (i.e. duration of hydration of plant tissue), frequency and velocity of desiccation, and duration of the dehydrated state. Due to its highly expressed mechanisms of stress tolerance – constitutive levels of raffinose and dehydrins in submerged leaves, most sensitive response of ABA to dehydration among all resurrection spermatophytes examined, contractive tracheids and cytorrhysis, velamen radicum – *Ch. intrepidus* is able to respond to the frequent and fast desiccation events, which are characteristic for shallow pools, immediately without any damage to molecular, subcellular and cellular structures. On the other hand, rapid resumption of photosynthesis after rehydration by both submerged and floating leaves (WÖITKE et al. 2004) and utilisation of urine, which is especially abundant in the pool water at the beginning of the growing season, and of amino acids during periods of ammonium shortage enable the plant to capture resources effectively. Nevertheless, the short period of resource availability and acquisition and the high metabolic costs associated with constitutive tolerance mechanisms limit the habitat of *Ch. intrepidus* to the most extreme of all resurrection plants. Since the bottom of most pools is rather flat, maximum water level is more or less uniform for the whole pool area. Thus the low spatial heterogeneity causes only one of the two Scrophulariaceae resurrection plants to occur in any one pool in most of the cases. Only in pools with a more concave bottom, *L. grandiflora* grows in the centre with a higher water table, whereas *Ch. intrepidus* is restricted to the less deep periphery of the pools (cf. Fig. 2a).

Table 1. Results of logistic regressions describing the distribution of *Chamaeigas intrepidus* and *Limosella grandiflora* in rock pools on granite outcrops in Central Namibia.

The equation for the logistic regression model is:

$$\log_e(P/(1-P)) = \beta_0 + \beta_1 * x$$

with P = probability of occurrence

x : site factor

β_0, β_1 : intercept and regression coefficient assigned to the independent variable x

SE indicates standard error for the estimates, t-ratio and significance levels (p-value) are only given for the regression coefficients. Sensitivity and specificity refer to the percentage of correctly classified occurrences and non-occurrences, respectively (both at $p = 0.05$).

	β_0	SE	β_1	SE	t-ratio	p-value	sensitivity	specificity
<i>Chamaeigas intrepidus</i>								
maximum water level	2.32	0.423	-0.176	0.053	-3.32	0.001	78.6	32.5
depth of sediment	0.783	0.261	1.611	0.815	1.98	0.048	78.4	26.4
mineral proportion in sediment	0.570	0.266	0.557	0.194	2.862	0.004	77.8	29.2
conductivity	0.125	0.508	-0.007	0.032	-0.205	0.837	50.9	49.1
<i>Limosella grandiflora</i>								
maximum water level	-5.91	1.08	0.352	0.088	3.994	<0.001	36.3	96.0
depth of sediment	-3.09	0.496	0.089	0.575	0.154	0.877	4.5	95.5
mineral proportion in sediment	-1.14	0.406	-42.5	167.8	-0.253	0.800	24.2	95.2
conductivity	-2.75	0.970	0.015	0.056	0.259	0.795	7.4	92.7

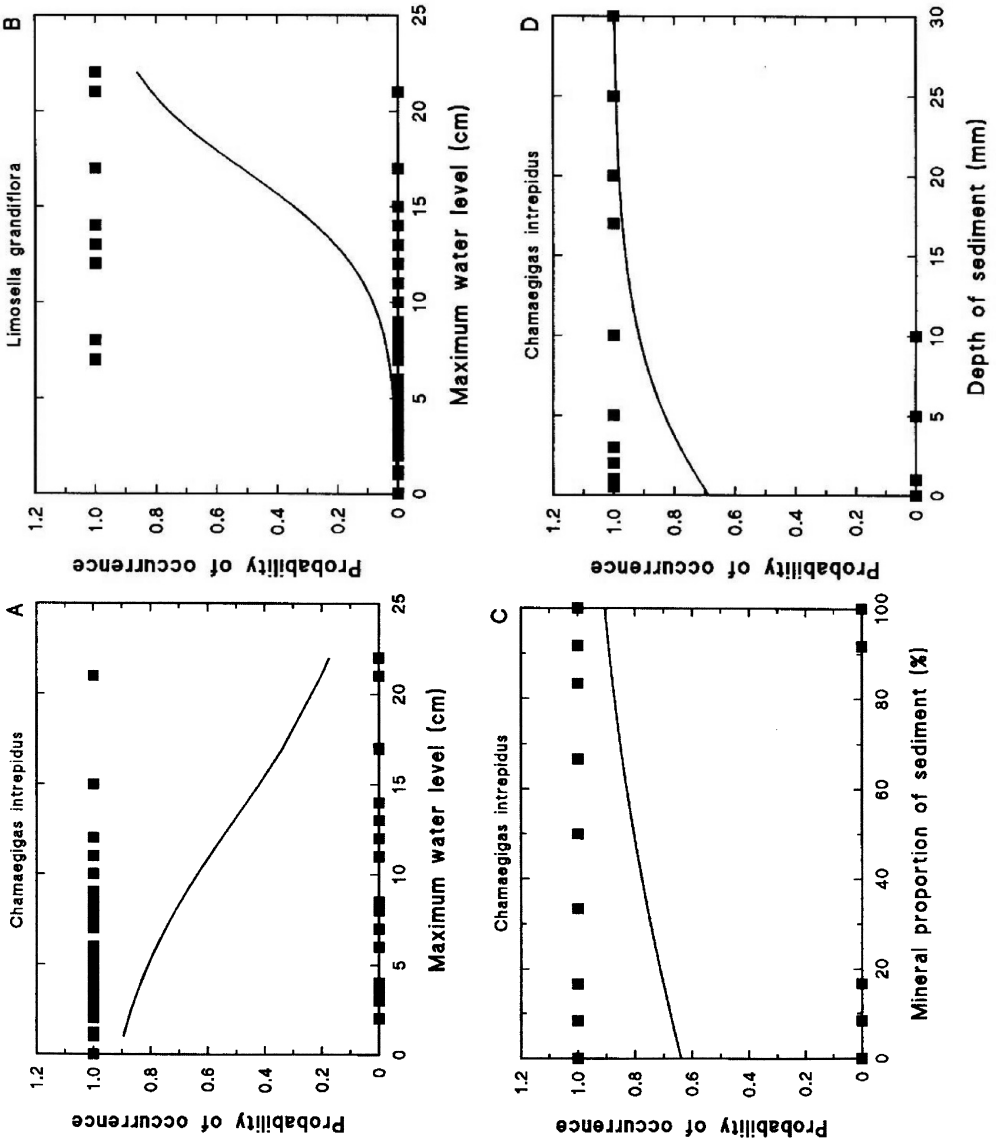


Fig. 3. Observed (presence/absence data points) and predicted probabilities (logistic regression curves from habitat models, cf. Table 1) for the occurrence of aquatic resurrection plants in ephemeral rock pools in Central Namibia: Fig. 3a: Occurrence of *Chamaeigigas intrepidus* as dependent on maximum water level of the pools. Fig. 3b: Occurrence of *Limosella grandiflora* as dependent on maximum water level of the pools. Fig. 3c: Occurrence of *Ch. intrepidus* as dependent on the mineral proportion of the sediment in the rock pools. Fig. 3d: Occurrence of *Ch. intrepidus* as dependent on the depth of the sediment in the rock pools.

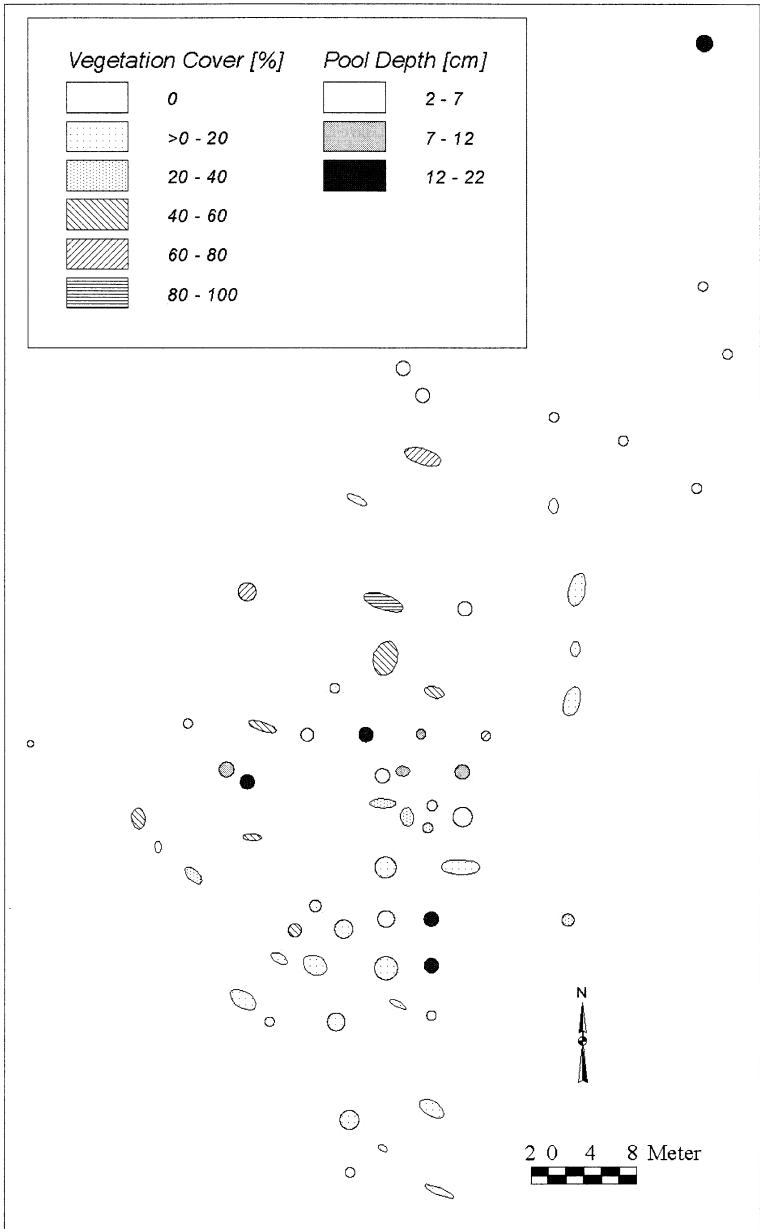


Fig. 4. Distribution, pool depth and cover by *Chamaegigas intrepidus* in ephemeral rock pools on a granite outcrop on the farm Otjua (Omaruru District, Namibia). Location of pools was measured with a global positioning system (Projection: Universal Transverse Mercator, Horizontal Datum: WGS 84) and by tape measure plus compass.

6. Life history traits related to stochastic stress events and habitat isolation

According to WILLIAMS (1985) the environmental instability of temporary waters is a stressor on its own, in addition to all the individual stress factors which act synergistically. Thus, even within the wet season the occurrence and duration of drought stress is not predictable. In contrast to *L. grandiflora* which grows in the more stable deeper pools, dehydration tolerance of *Chamaegigas* leaves is a major adaptive trait in view of the highly variable hydration state of the plant. This dehydration tolerance was previously ascribed to submerged and immature floating leaves only (GAFF & GIESS 1986). However, during short intervals of desiccation and rehydration within a rainy season, chlorophyll fluorescence response was not different for submerged and fully expanded floating leaves, indicating that also mature floating leaves are tolerant to tissue dehydration (WOITKE et al. 2004). In view of the high N content of floating leaves, this behaviour can be interpreted as a mechanism to save possibly limiting nutrients by maintaining the tissue. Thus, longevity of various plant organs and retention time for nutrients is a major mechanism of adaptation to stochastically fluctuating resource availability.

Apart from temporal unpredictability, spatial arrangement of suitable habitats for *Chamaegigas* within the (semi-)arid landscape matrix is rather irregular. While several pools may be clumped on a single rock outcrop, distribution patterns of these inselbergs are highly random. Although the plant is able to spread vegetatively, we found the major proportion of genetic diversity within the local population in single pools, in accordance with previous reports on a high genetic diversity within clonal plants (ELLSTRAND & ROOSE 1987, WIDÉN et al. 1994). This high genetic variability in spite of the restricted geographic range and specific habitat requirements of the plant agrees with the predominantly outcrossing breeding behaviour and seed dispersal mode. On the outcrop depicted in Fig. 4 average distance between pools populated by *Ch. intrepidus* was 6.9 m (median for nearest neighbour distance, minimum 3.0 m, maximum 54 m, $n = 36$). Moreover, the dispersion index DI (variance of pool number per random square/average pool number per random square; KREBS 1989) indicates a slightly aggregated pattern for pools populated by *Ch. intrepidus* ($\chi^2 = 14.4$, $df = 6$), whereas distribution pattern of all pools on the rock outcrop is clearly random ($\chi^2 = 11.0$, $df = 6$). In view of the short distances between and the aggregated distribution pattern of *Chamaegigas* pools, the high abundance of pollinating beetles and wild bees and seed dispersal by wind accomplish a high gene flow both within and among pools on a single inselberg, decreasing genetic subdivision within populations (LOVELESS & HAMRICK 1984). On the other hand, minimum distance between inselbergs (ca. 10 km for the sites shown in Fig. 1) is well beyond flight distance of insects pollinating *Ch. intrepidus*, and also seed dispersal by birds or animals visiting the pools for drinking water should be rare. This is evident from the high level of genetic differentiation on the site level and the non-significant cor-

relation between genetic differentiation and geographic distance which suggests that isolation by distance does not occur. Rather, the patchy spatial distribution of suitable habitats within a landscape matrix with low connectivity drastically reduces gene flow between inselbergs and enhances differentiation between *Chamaeigigas* populations. If selection is excluded, both genetic drift and possibly high mutation rates due to excessive UV radiation may be the dominant factors for both interpopulation and intrapopulation genetic structure. Since genetic variation is often correlated to reproductive traits like number of seeds per plant (SCHMIDT & JENSEN 2000), stochastic genetic processes within *Chamaeigigas* populations may maintain both a high genetic variability and abundance of propagules, at least in the long-term evolutionary perspective, even when recruitment is very low in each generation. In five years of study at Otjua, only in one year a large number of seedlings (> 100) was found in one pool which, however, did not survive the dry winter season. However, in 2002 on the farm Okapekaha similar large numbers of seedlings were found in two pools, whose fate, however, is not known.

Presumably rare events of sexual reproduction found for *Ch. intrepidus* in the pools studied suggest that the major mechanism of adaptation to the low predictability of periods favourable for plant growth and reproduction is the persistence of existing genets. Long-lived perennial species with a stable population size at a high level show low impact of genetic drift, i.e. a high resistance to chance fluctuations in gene and genotype frequencies (HAMRICK et al. 1979), preventing *Chamaeigigas* populations from becoming genetically impoverished. Traits like desiccation tolerance of both submerged and floating leaves, and the maintenance of the physical root structure even in the dehydrated state allow for a high longevity and retention time of nutrients, reducing the risk of nutrient loss. On the other hand, *Ch. intrepidus* is able to capture resources during temporarily favourable conditions immediately. This high physiological plasticity in combination with a conservative strategy with respect to resources is characteristic for stress-tolerant plants (GRIME 2001). For seasonal rock pools in the savanna zone of the Ivory Coast the dominating life form are r-selected annual species (KRIEGER et al. 2000). For the aquatic resurrection plants occurring in ephemeral pools in the semi-arid region of Central Namibia, especially *Ch. intrepidus* at the most extreme habitats, the duration of favourable periods may be too short to complete a therophytic life cycle. Therefore, these aquatic macrophytes do survive the dramatic environmental stochasticity with its low predictability of the occurrence and duration of favourable versus unfavourable periods by being a slowly growing, long-lived poikilohydric stress tolerator.

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