

Somatic Polymorphism Variation in *Crotalaria retusa* L. Seeds

Paulo Sérgio de Figueiredo, Natale M. Lindoso Silva

Department of Biology, Federal University of Maranhão State, São Luís, Brasil

Email: paulosfigueiredo@uol.com.br

How to cite this paper: de Figueiredo, P.S. and Silva, N.M.L. (2018) Somatic Polymorphism Variation in *Crotalaria retusa* L. Seeds. *American Journal of Plant Sciences*, 9, 46-59.

<https://doi.org/10.4236/ajps.2018.91005>

Received: October 27, 2017

Accepted: January 8, 2018

Published: January 11, 2018

Copyright © 2018 by authors and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

The article describes the somatic polymorphism in *Crotalaria retusa* L. seeds. Each individual may produce yellow dormant seeds, brown quiescent seeds and unviable dark brown ones. Therefore, regarding physiology it is a dimorphism. We show that autogamy reduces dimorphism, favoring the formation of dormant seeds. However, the significant variation in dimorphism is seasonal as consequence of increased viability of dormant seeds under drier weather conditions. The variation is a phenotypic response to changes of humidity in the environment during plant reproduction, a mechanism inducing the prevalence of dormant seeds in the dry season and quiescent seeds during the rainy season. The seasonal alternation between dormancy and quiescence in seasonally dry environments has an apparent adaptive value. The chromatic polymorphism increases on the more humid coast and during the rainy season, due to increased mortality of dormant seeds under wetter weather conditions. Unviable seeds accumulate oxidized phenols in their seed coat that possibly act as induced chemical defenses. Its proportion increases with humidity, accentuating polymorphism, also in response to the increase in the predation rate by larvae of *U. ornatrix* during the rainy season.

Keywords

Somatic Polymorphism, Phenols, Seed Coat Impermeability, Chemical Defenses, Predation

1. Introduction

Crotalaria retusa L. (Fabaceae, Faboidea) is an annual subshrub leguminous, native from Asia, Africa and Australia that has been actively introduced in many tropical and subtropical regions of the world to be used in agroforestry systems [1]. *Crotalaria retusa* is self-compatible [2], efficiently propagated by seeds and is

tolerant to a wide range of environmental moisture variation [3], characteristics that led it to become a noxious weed with Pantropical distribution [4]. The species is particularly abundant in the Northeast Atlantic coast of Brazil [2] and it is frequently found in the disturbed habitats inland as well as on the littoral. The coastal populations are common in primary dunes near a high tide line, local where they exhibit the characteristic prostrated habit, which is considered a typical adaptation to the environmental extremes of the ecosystem [5].

Although *C. retusa* plants are annual and have a short life-cycle, of less than 5 months, the growth and reproduction of the populations approach the continuum in the Northeast region of Brazil, due to 5 to 6 asynchronous annuals life-cycles [2]. Asynchrony is generated by somatic polymorphism: A dormancy mechanism in which a single individual produces different morphophysiological types of seeds, and that is restricted to a small number of families such as Fabaceae, Asteraceae, Chenopodiaceae and Poaceae [6] [7]. Inducing the formation of soil seed bank and distributing the germination in time [8] [9], the polymorphism confers resilience and multiple plant establishment opportunities [10] [11], remarkable traits for invading plants, adapting them to the extreme instability of early successional habitats in anthropic areas [12].

Polymorphism is apparently more accentuated in coastal populations, suggesting an ecotypic adaptation to the environment. The somatic polymorphism is a phenomenon relatively common in pioneer species of the coastal dunes [5] [13] and it has been interpreted as an adaptation to greater hostility and variability of environmental conditions that characterize the habitat [14] [15]. Since autogamy tends to reduce the genetic polymorphism [16], polymorphic changes between habitats in self-compatible species may not reflect genetic differences between populations, but differences in selfing rates. The effect of autogamy in seed somatic polymorphism is still unknown and it is essential to evaluate its effect before characterizing any ecotypic difference.

In *Crotalaria retusa* seeds, somatic polymorphism is visually identified as a color gradient in the seed coats, ranging from yellow to brown which appear to be associated with differences in the degree of dormancy by seed coat impermeability to water: physical dormancy [17]. The chemical and anatomic changes that alter the seed coat permeability are not completely understood but it is considered that impermeability is due to the final thickening, by the suberization or lignifications of columnar cell walls in seed coats [17] [18]. Impermeability is acquired in the final maturation stages being particularly influenced by the relative air humidity during the dehydration of orthodox seeds [19] [20], whereas low humidity favors impermeable coats [7].

The chromatic polymorphism, with tones varying from yellow to brown seeds, is attributed to the differential oxidation of phenolic compounds in the seed coats, which are concentrated as polymers that become cross-linked to wall components during seed maturation [21] [22]. This oxidative browning has been positively correlated with thicker cell walls that provide greater mechanical re-

straint and reduced permeability to water and/or gases, ways in which the seed coat exerts its germination restrictive action [22] [23] [24].

Although the oxidation of phenols in the seed coat is correlated with impermeability, phenols are secondary metabolites ubiquitous in seeds and considered as the most important chemical defense compounds against pathogens and herbivores [25] [26]. Phenolic compounds inhibit the enzymatic activity in fungi, bacteria and virus, as well as slow growth and reproduction in insects [27] [28]. The high concentration of pyrrolizidine alkaloids makes the *Crotalaria* ssp seeds toxic leading to fewer natural predators. The most notable among these are *Ute-theisa ornatrix* (Lepidoptera, Arctiidae) aposematic larvae, whose seed-based diet confers resistance against the predation of the eggs by ants, and of larval and adult forms by spiders [29] [30].

The predation of reproductive structures has a profound effect on the evolution of adaptive response [31] and the spatio-temporal variation in the predation rate can influence both selection on plants traits and its population dynamic [32]. If the phenolic compounds in *C. retusa* seeds are related to chemical defense, the somatic polymorphism variations of the seeds of coastal and inland populations can also be consequences of biotic pressure, such as the predation of seeds by *U. ornatrix* larvae.

The aim of this work was to characterize the polymorphism in *C. retusa* seeds and the role that autogamy plays in the phenomenon. To understand the apparent rise of polymorphism on the coastal regions, we compared the differences in polymorphism between littoral and inland populations, as well as its seasonal variation. The results were correlated with the seasonal variation of humidity in the environment and seed predation rates.

2. Materials and Methods

2.1. Climate of Study Location

The research was realized in urbanized areas in São Luís, MA (2°31'34"S, 44°12'32"W), in the Northeast Brazil. Situated in the equatorial region, in the transition from the Amazon forest to the semi-arid region, it is found in Aw climate or tropical hot and wet with summer and autumn rains, according to the Köppen classification. With relatively constant photo and thermoperiods (around 12 hours of light per day and an average annual temperature of 32°C/26°C) the region displays pronounced seasonality in rainfall. The "rainy season" extends from January to June and is characterized by precipitation between 1.600 mm to 2.400 mm, with a water surplus over 1.000 mm. A period of drought between July and December, with monthly precipitation below 100 mm and a moderate water shortage in the soil, characterizes the "dry season".

2.2. Polymorphism Characterization: Determination of the Mass, Humidity Percentage and Seed Water Absorption Rate

The seed mass and its humidity percentage were estimated for each seed coat

color class in randomly collected mature seed samples from 20 individuals. The data were obtained by weighing seeds, before and after oven drying at 100°C for 72 hours. The water absorption curve was obtained by periodically weighing, for 16 hours, samples of hydrated seeds in distilled water. The data were expressed as the percentage of water absorbed in relation to the seeds initial water percentage. The sample was composed of three replicates of ten seeds each.

2.3. Polymorphism Characterization: Germination

The seeds, separated per coat coloration class, were set to germinate in Petri dishes with two sheets of filter paper, permanently humidified with distilled water. The experiment was performed with four replicates of 50 seeds each. Germination tests were conducted under standard laboratory conditions, with an average daily temperature of 32°C/26°C and diffuse natural light.

2.4. Spatio-Temporal Variation in Polymorphism and in Predation Rates

Spatio-temporal variation in polymorphism and in predation rates was estimated between the habitats, on the coast and inland, in two seasons: at the height of the dry season in October and at the height of the rainy season in March. In each season there were four inland and four coastal populations sampled, which were selected as grouped individuals occurring spontaneously or inland, at least 5 km from the coast, or in the primary dunes along the urbanized coast. The degree of polymorphism and the predation rates were estimated by comparing the numbers of seeds in each seed coat coloration class and the number of fruits preyed on, in a random sample of 20 fruits per individual, with a total of ten individuals per population. The predation was identified through a hole in the fruit, about 3 mm in diameter, caused by the outbreak of *Utheteisa ornatrix* L. (Lepidoptera: Arctiidae) adults and through the remains of fragmented seeds.

2.5. The Effect of Autogamy in Polymorphism

The influence of pollen origin on seed color was estimated through the technics of breeding systems analyses. The experiment was performed in two periods: at the height of dry period in October and at the height of rainy period in March. The plants were cultivated in gardens in the University campus and submitted manual self-pollination, manual cross-pollination and natural pollination (open pollination). Artificial pollination was performed by isolating emasculated flowers in bags on pre-anthesis phases. For open pollination the flowers were tagged and monitored. The fruit set was recorded after full development and the number of seeds in each color class, the fruit/flower rate and the number of seeds per fruit were determined.

2.6. Chemical Defenses versus Predation

The number of brown seeds (brown, dark brown and black seeds) in the 16 pop-

ulations previously sampled was used as an index of chemical defenses, and its proportion correlated with the rate of predation.

2.7. Statistical Analysis

The mass, humidity percentage and germination data were compared by One-way ANOVA followed by the test of minimum significant difference of Fisher [34]. The variation in the degree of polymorphism and in predation rate was compared by Two-way ANOVA, the decomposing variance as an effect attributed to the season (temporal variation) or to the habitat (spatial variation). As the same way, Two-way ANOVA was used in the breeding system analyses to compare differences between self and cross pollination in the dormancy/quiescence ratio ($R = D/D + Q$). For this particular experiment, the variance was decomposed as an effect due to either the type of pollination or to the season. Proportions were arcsine transformed before statistical analysis to ensure homogeneity of variance (non-transformed data appear in all figures). The correlation between the proportion of dark seeds in the populations (brown, dark brown and black) and the predation rate was obtained by simple linear regression, estimating the Pearson coefficient. In all of the tests, the significance level adopted was 5% [34].

3. Results

3.1. Polymorphism Characterization

The polymorphism in *C. retusa* seeds is characterized as a gradient from yellow to very dark brown seed coat, denominated as black seeds here (Figure 1).

Table 1 shows that ripe seeds differ in regards to their mass and percentage of humidity. Those of intermediate coloring (dark yellows and browns) exhibit the greatest mass ($F = 119$, $p < 0.01$), while the darkest seeds (dark brown and black) are the smallest ones ($F = 140.3$, $p < 0.001$). The yellow seeds (yellows and dark

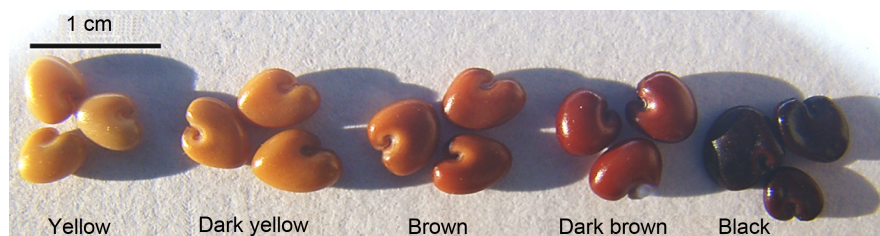


Figure 1. Chromatic polymorphism in *C. retusa* L. seeds.

Table 1. Weight and moisture content of *Crotalaria retusa* L. polymorphic seeds.

Seed Coat Color	Yellow	Dark Yellow	Brown	Dark Brown	Black
Mass (mg)	13.2 ^B ± 0.6	15.37 ^A ± 0.5	15.58 ^A ± 1.8	11.47 ^C ± 0.2	5.5 ^D ± 0.1
% of humidity	9.49 ^a ± 0.2	9.56 ^a ± 1.4	14.65 ^b ± 1.2	12.48 ^b ± 0.7	22.3 ^c ± 2.0

Upper case letters compare seed mass and the lower cases compare the degree of humidity. Different letters indicate statistically distinct values ($p < 0.05$).

Yellows) have a matching degree of moisture ($F = 0.002$, $p = 0.95$), which is significantly less than the brown, dark brown and black seeds ($F = 69$, $p < 0.001$).

The chromatic gradient, from yellow to brown, is directly proportional to the seed coat permeability degree, with yellow seeds exhibiting seed coat impermeability, not absorbing water or absorbing it very slowly, after 16 hours of continuous hydration. The brown seeds have the moderately permeable coat, absorbing approximately 15% of water during the same period, while the dark brown and black seeds have a permeable coat, absorbing between 30% and 35% of humidity, respectively, after 6 hours of continuous hydration (**Figure 2**).

The yellow seeds (yellow and dark yellow with impermeable seed coat) are dormant, exhibiting a low final germination percentage of circa 20% after 27 days. The brown seeds (with moderately permeable seed coat), are quiescent, reaching nearly 70% of germination in the same period. The darkest seeds (dark brown and black with permeable seed coat) don't germinate at all (**Figure 3**). The later produce an exudate and an odor associated with rotting, during the first 72 hours of hydration, indicating that these seeds are unviable.

3.2. Spatio-Temporal Variation in Polymorphism and Predation Rates

The analysis of **Figure 4** reveals the prevalence of temporal variation over the spatial in the degree of polymorphism (the relative proportion of the different forms). The polymorphism increases during the rainy season due to the significant reduction in the proportion of dormant seeds (yellow and dark yellow, $F =$

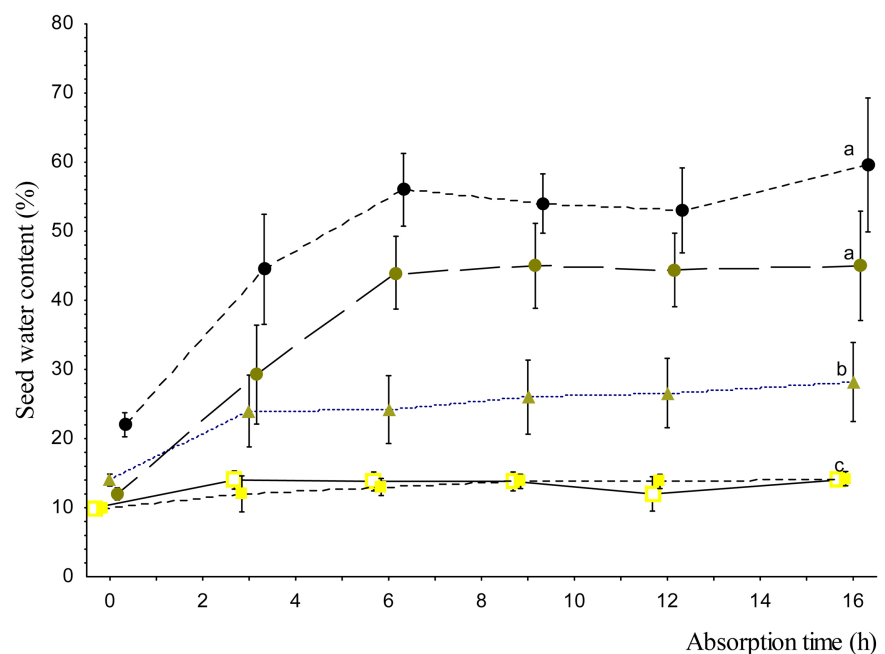


Figure 2. Water absorption by *Crotalaria retusa* L. polymorphic seeds. □ Yellow seeds; ■ Dark yellow; ▲ Brown; ● Dark brown; ● Black. Vertical bars denote the standard error. Letters compare the final percentage of absorbed water. Different letters indicate statistically distinct values ($p < 0.05$).

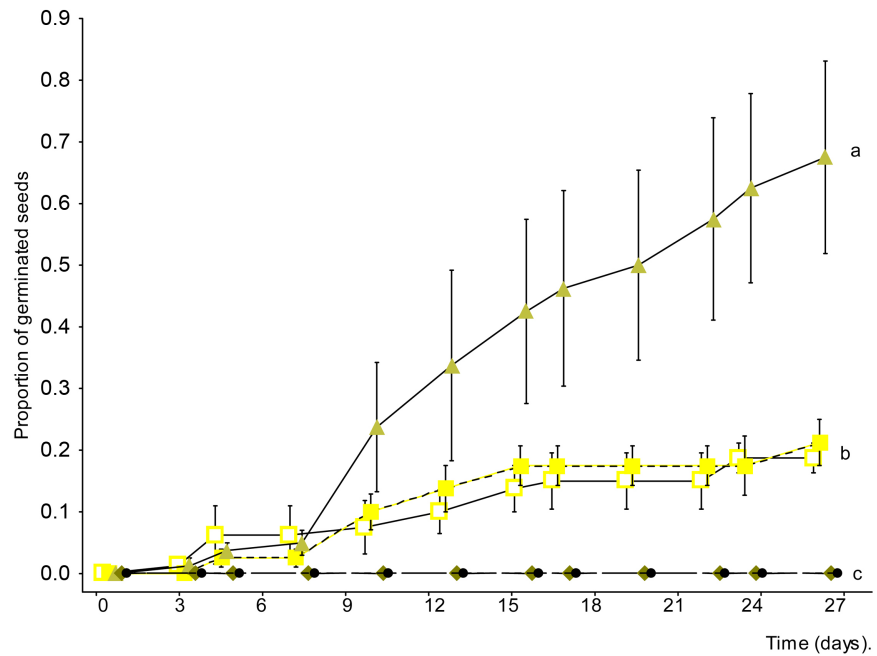


Figure 3. Germination of the *Crotalaria retusa* L polymorphic seeds. □ Yellow seeds; ■ Dark yellow; ▲ Brown; ● Dark brown; ● Black. Vertical bars denote the standard error. Letters compare the final proportion of germinated seeds. Different letters indicate statistically distinct values ($p < 0.05$).

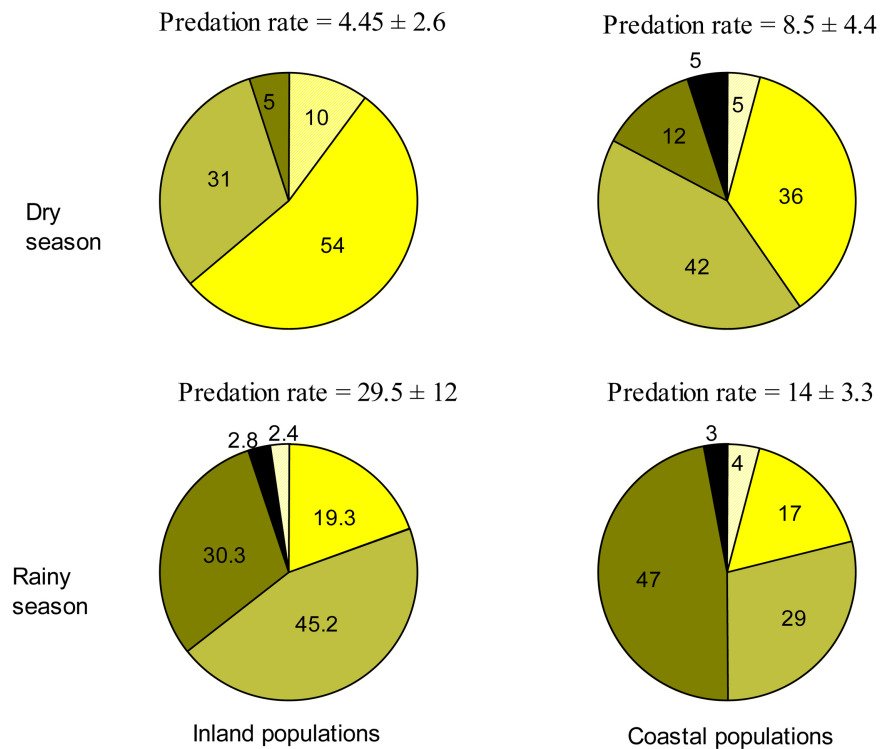


Figure 4. Spatio-temporal variation in polymorphism degree (relative proportion of different forms) and in the predation rate (% ± σ) in *Crotalaria retusa* L. seeds. ▨ Yellow seeds; ■ Dark yellow; ■ Brown; ■ Dark brown; ■ Black. Numerical values in the figure indicate the percentage of different seed color class.

17.88, $p = 0.001$) and significant increase in the proportion of unviable seeds (dark brown and black, $F = 24.62$, $p < 0.0001$). The proportion of quiescent seeds (brown) remains constant ($F = 0.50$, $p = 0.48$).

The differences between the inland and coastal habitats are marginally significant, with the coastal populations producing a higher proportion of unviable seeds in both seasons ($F = 6.15$, $p = 0.028$), but only showing a higher degree of polymorphism than the inland populations during drought.

The predation rates also exhibit greater temporal variation than spatial variation, being significantly greater in the rainy season ($F = 20.68$, $p < 0.0001$). Between the habitats, the predation was significantly greater in coastal populations in the dry season, however significantly greater in the inland populations during the rainy season ($F = 8.39$, $p = 0.013$).

3.3. The Effect of Autogamy on Polymorphism

A significant effect of autogamy can be observed through manual pollination, in **Figure 5**. Autogamy reduces the polymorphism by favoring the formation of hard seeds, while cross-pollination keeps significant proportions of quiescent seeds, maintaining the polymorphism (Pollination effect $F = 4.29$, $p = 0.045$). This effect remains in both periods (season effect not significant, $F = 2.93$, $p = 0.095$), despite the high seed mortality in the rainy season. In all treatments, the fruit/flower ratio (R) and the number of seeds per fruit (S) were higher in the dry season.

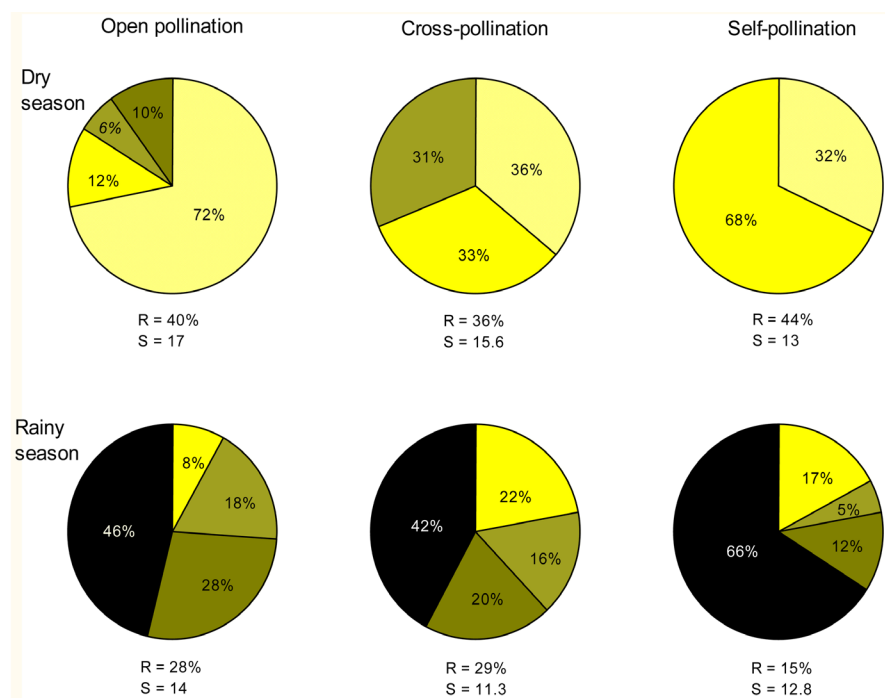


Figure 5. The effect of autogamy on somatic polymorphism in *Crotalaria retusa* L. seeds. Yellow seeds; Dark yellow; Brown; Dark brown; Black. R = fruit/flower ratio; S = number of seeds/fruit. Numerical values in the figures indicate the percentage of seeds produced per color class.

3.4. Predation Rates versus Oxidation of Phenols in Seed Coat

The predation rate in the populations is directly proportional to the concentration of oxidized phenols ($p = 0.004$) in the seeds (Figure 6). The variation of the predation rate explains approximately 50% of the variation in the concentration of oxidized phenols ($r^2 = 0.51$).

4. Discussion

In the seeds of *Crotalaria retusa* L. the chromatic gradient, from yellow to brown, correlates directly with the tegument permeability degree. Impermeability occurs in the yellow seeds, which are more dehydrated in maturation. This fact reinforces the hypothesis about the influence of the degree of dehydration in the acquisition of impermeability on the so-called “hard seeds” [19] [20], but contradicts that of impermeability as a consequence of phenol oxidation in the seed coat during dehydration, as suggested by several authors [21] [22] [23]. The developmental series originating dormant yellow seeds and quiescent brown ones in the same fruit cannot be a case of heterocrony as defined by Silvertown [7], actually representing distinct metabolic pathways.

Since dark brown and black seeds are inviable, the *C. retusa* polymorphism, regarding germination physiology, is in fact a dimorphism: yellow seeds with physical dormancy and quiescent brown seeds. The dark yellow and brown forms stand out both due to their greater mass, a characteristic of unequivocal adaptive value [35] [36] [37], as well as by their quantity, comprising between 50% and 80% of all seeds produced. Autogamy, whose frequency increases with the decrease of the pollinators [38] favors dormant forms, reducing dimorphism. Thus, some variation in the proportion of dormant and quiescent forms among

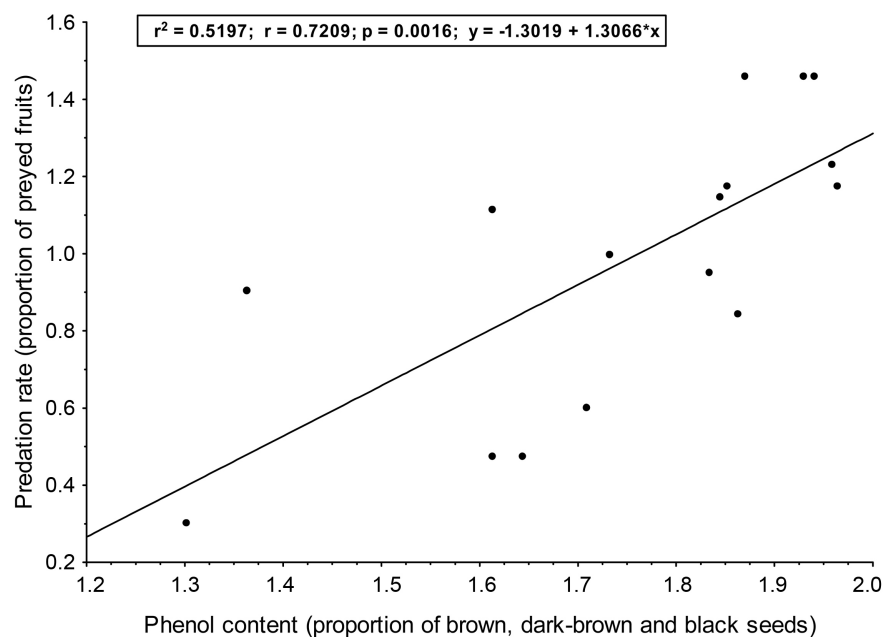


Figure 6. Correlation between phenol content and predation rate.

populations is due to the variation in the frequency of the pollinator. It is interesting to consider that quiescence, favoring immediate colonization, is maximal when the pollinators are present, while dormancy, favoring resilience and sporadic and irregular colonization, prevails in their absence. This suggests strategy selection during colonization episodes.

Notwithstanding the effect of autogamy, the most significant variation in this dimorphism is seasonal, due to the increase in the proportion of dormant seed (viability of dormant seeds) with an increase in environmental aridity. This increase of “hard seeds” in the drought is consistent with the effects of low relative humidity in the formation of impermeable coat [7] and with the hypothesis of the evolution of physical dormancy as one adaptation to environmental aridity [17]. In seasonally dry environments the increase in viability of dormant seeds with aridity determines the prevalence of dormancy in the drought and quiescence during the rainy period. The seasonal alternation between quiescence and dormancy in these environments has an apparent adaptive advantage, allowing the species to react opportunistically to seasonal rains. This seasonal alternation is even more pronounced in the native invaders *Mimosa pudica* L. and *Desmodium incanum* L. (unpublished data), which exhibit seed dimorphism similar to that of *C. retusa* L.

While the seasonal variation in somatic dimorphism has a clearly adaptive nature, the variation in color polymorphism appears to be a simple environmental stress effect caused by the increased humidity. The polymorphism stands out during the rainy season and on the humid coast during drought, due to the increase in the unviable dark brown seeds: a consequence of mortality of dormant seeds with increasing humidity. The prevalence of temporal variation over spatial variation indicates that the plasticity is phenotypic, which leads us to reject the hypothesis expressed in the introduction, that the largest polymorphism in the littoral could represent an ecotypic adaptation. As the polymorphism stands out due to the increase in seed mortality, its adaptive value is questionable.

One aspect that should be considered is that the unviable seeds, as well as the quiescent ones, concentrate oxidized phenols in the seed coat. The oxidative browning, in which the phenols are oxidized to o-quinones that react by producing brown polymers [39] [40], is observed during the aging of several plant tissues and it is associated with the production of antimicrobial agents and oxygen reactive species, which act as chemical defenses against predators and pathogens [24] [40].

In the *C. retusa* populations, the positive correlation between the accumulation of oxidized phenols and the rate of seed predation suggests induced chemical defense [14] which, particularly concentrated in unviable seeds, increase as a response to the increase of the predation rate by *U. ornatrix* larvae. Predation is a strong selective force; a single developing larva consumes all seeds in the fruit and predation, which is low in drought, increases significantly during the rainy season, reaching up to 80% of all seeds produced in certain populations. The

seasonal increase of chemical defenses in seeds is accompanied by a great reduction in the number of seeds produced (reduction in the fruit/flower ratio and seed/fruit ratio) resulting in an offer of a diet with fewer, smaller seeds (dark brown seeds make up most of the unviable seeds and it has 70% of the whole seed mass), and rich in phenols. This restricted diet can be an efficient control mechanism of larval populations of *U. ornatrix* L. during the rainy season.

Jacobi *et al.* [2] considered that the predominance of *C. retusa* in the coastal landscape in the northeastern Brazil seems to fundamentally depend on the success in attracting its main pollinators: native bees *Xylocopa* sp. which exhibit wide geographical distribution in the humid tropics. The reduction in the viability of seeds under wetter weather conditions, coupled with a reduction in fruit/flower ratio, seed/fruit ratio and an increase in predation rate implies that reproduction is optimal in the dry season, which should favor the adaptation of the species to the Semi-arid and seasonally dry Atlantic coast, limiting its expansion to the “always humid” western Amazon.

5. Conclusions

In somatic dimorphism dormant and quiescent seeds of *C. retusa* L. develop in the same fruit by distinct metabolic pathways, not by heterocrony. Although some variation in somatic dimorphism between populations of *C. retusa* L. is generated by the variation in the rate of self-fertilization, with autogamy favoring dormant forms, this variation is mainly seasonal, a plastic response (phenotypic plasticity) to the change of humidity in seasonally dry environments. It is a consequence of the increase in viability of dormant hard seeds under arid climate; which induces the predominance of dormancy in drought, quiescence during the rainy season and equitable proportions of the two forms on the wettest coastline during drought.

The variation in chromatic polymorphism is also predominantly seasonal as a consequence of dormant seed mortality under wetter weather conditions. In viable seeds, which together with the quiescent ones concentrate oxidized phenols on seed coat, may constitute chemical defenses against predation by larvae of *U. ornatrix* L., whose activity increases significantly in the rainy season.

Acknowledgements

Our thanks to the zoologists Dr. Vitor O. Becker and Dr. Marcio Romero for identifying *Utetheisa ornatrix* L. larvae and adults. Thanks also to the biologists karla J. C. Bezerra and Josycarla L. Santos by the indispensable support in the collection of initial data. Also to our friend and environmentalist Kiko from the admirable NGO, Orla Viva, who patiently recorded the relative humidity data in the dunes. We would also like to thank Dr. Emília C. Girnos for revising the manuscript and for the constructive hours of discussion.

References

- [1] Brunner, B., Martínez, S., Flores, L. and Morales, P. (2013) *Crotalaria*. Hoja Infor-

- mativa. Proyecto de Agricultura Orgánica Z-NRCS-007/Z-208, Puerto Rico: Estación Experimental Lajas.
- [2] Jacobi, C.M., Ramalho, M. and Silva, M. (2005) Pollination Biology of the Exotic Rattle Weed *Crotalaria retusa* L. (Fabaceae) in NE Brazil. *Biotropica*, **37**, 357-363. <https://doi.org/10.1111/j.1744-7429.2005.00047.x>
- [3] Rojas-Sandoval, J. and Acevedo-Rodríguez, P. (2015) *Crotalaria retusa* (rattleweed). <http://www.cabi.org/isc/datasheet/87408>
- [4] USDA (2006) National Genetic Resources Program. Germplasm Resources Information Network—(GRIN) [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland.
- [5] Waisel, Y. (1972) Biology of Halophytes. Academic Press, New York.
- [6] Baskin, J.M. and Baskin, C.C. (1976) Germination Dimorphism in *Heterotheca subaxillaris* var. *subaxillaris*. *Bulletin of the Torrey Botanical Club*, **103**, 201-206. <https://doi.org/10.2307/2484679>
- [7] Silvertown, J.W. (1984) Phenotypic Variety in Seed Germination Behavior: The Ontogeny and Evolution of Somatic Polymorphism in Seeds. *American Naturalist*, **124**, 1-16. <https://doi.org/10.1086/284249>
- [8] Baskin, C.C. and Baskin, J.M. (1988) Germination Ecophysiology of Herbaceous Plant Species in a Temperate Region. *American Journal of Botany*, **75**, 286-305. <https://doi.org/10.2307/2443896>
- [9] Bewley, J.D. and Black, M. (1994) Seeds: Physiology of Development and Germination. 2nd Edition, Plenum Press, New York. <https://doi.org/10.1007/978-1-4899-1002-8>
- [10] Thompson, P.A. (1981) Ecological Aspects of Seed Germination. In: Thompson, J.R., Ed., *Advances in Research and Technology of Seeds*, Centre for Agricultural Publishing and Documentation, Amsterdam, 9-42.
- [11] Ewing, M.A. (1999) Annual Pasture Legumes: A Vital Component Stabilizing and Rehabilitating Low-Rainfall Mediterranean Ecosystems. *Arid Soil Research and Rehabilitation*, **3**, 327-342. <https://doi.org/10.1080/089030699263221>
- [12] Baker, H.G. (1974) The Evolution of Weeds. *Annual Review of Ecology and Systematics*, **5**, 1-24. <https://doi.org/10.1146/annurev.es.05.110174.000245>
- [13] Li, W.Q., Liu, X.J., Khan, M.Aa. and Yamaguchi, S. (2005) The Effect of Plant Growth Regulators, Nitric Oxide, Nitrate, Nitrite and Light on the Germination of Dimorphic Seeds of *Suaeda salsa* under Saline Conditions. *Journal of Plant Research*, **118**, 207-214. <https://doi.org/10.1007/s10265-005-0212-8>
- [14] Kestring, D., Menezes, L.C.C.R., Tomaz, C.A., Lima, G.P.P. and Rossi, M.N. (2009) Relationship among Phenolic Contents, Seed Predation, and Physical Seed Traits in *Mimosa bimucronata* Plants. *Journal of Plant Biology*, **52**, 569-576. <https://doi.org/10.1007/s12374-009-9073-3>
- [15] Wang, L., Huang, Z., Baskin, C.C., Baskin, J.R. and Dong, M. (2008) Germination of Dimorphic Seeds of the Desert Annual Halophyte *Suaeda aralocaspica* (Chenopodiaceae), a C4 Plant without Kranz Anatomy. *Annals of Botany*, **102**, 757-769. <https://doi.org/10.1093/aob/mcn158>
- [16] Glémin, S., Bazin, E. and Charlesworth, I. (2006) Impact of Mating Systems on Pattern of Sequence Polymorphism in Flowering Plants. *Proceedings of the Royal Society of London B*, **273**, 3011-3019. <https://doi.org/10.1098/rspb.2006.3657>
- [17] Baskin, J.M., Baskin, C.C. and Li, X. (2000) Taxonomy, Anatomy and Evolution of Physical Dormancy in Seeds. *Plant Species Biology*, **15**, 139-152.

- <https://doi.org/10.1046/j.1442-1984.2000.00034.x>
- [18] Finch-Savage, W.E. and Leubner-Metzger, G. (2006) Seed dormancy and the Control of Germination. *New Phytologist*, **171**, 501-523. <https://doi.org/10.1111/j.1469-8137.2006.01787.x>
- [19] Williams, W.A. and Elliott, J.R. (1960) Ecological Significance of Seed Coat impermeability to Moisture in Crimson Subterranean and Rose Clovers in a Mediterranean-Type Climate. *Ecology*, **41**, 733-742. <https://doi.org/10.2307/1931807>
- [20] Lush, W.M. and Evans, L.T. (1980) The Seed Coats of Cowpeas and Other Grain Legumes: Structure in Relation to Function. *Field Crops Research*, **3**, 267-286. [https://doi.org/10.1016/0378-4290\(80\)90034-9](https://doi.org/10.1016/0378-4290(80)90034-9)
- [21] Debeaujon, I., Léon-Kloosterziel, K.M. and Koornneef, M. (2000) Influence of the Testa on Seed Dormancy, Germination, and Longevity in *Arabidopsis*. *Plant Physiology*, **122**, 403-413. <https://doi.org/10.1104/pp.122.2.403>
- [22] Finkelstein, R., Reeves, W., Ariizumi, T. and Steber, C. (2008) Molecular Aspects of Seed Dormancy. *Annual Review of Plant Biology*, **59**, 387-415. <https://doi.org/10.1146/annurev.arplant.59.032607.092740>
- [23] Moise, J.A., Han, S., Gudynaite-Savitch, L., Johnson, D.A. and Miki, B.L.A. (2005) Seed Coats: Structure, Development, Composition, and Biotechnology. *In Vitro Cellular & Developmental Biology-Plant*, **41**, 620-644. <https://doi.org/10.1079/IVP2005686>
- [24] Pourcel, L., Routaboul, J., Kerhoas, L., Caboche, M., Lepiniec, L. and Debeaujon, I. (2005) Transparent testa10 Encodes a Laccase-Like Enzyme Involved in Oxidative Polymerization of Flavonoids in *Arabidopsis* Seed Coat. *The Plant Cell*, **17**, 2966-2980. <https://doi.org/10.1105/tpc.105.035154>
- [25] Veldman, J.W., Murray, K.G., Hull, A.L., Garcia, J.M., Mungall, W.S., Rotman, G.B., Plosz, M.P. and McNamara, L.K. (2007) Chemical Defense and Thepersistence of Pioneer Plant Seeds in the Soil of a Tropical Cloud Forest. *Biotropica*, **39**, 87-93. <https://doi.org/10.1111/j.1744-7429.2006.00232.x>
- [26] Davis, A.S., Schutte, B.J., Iannuzzi, J. and Renner, K.A. (2008) Chemical and Physical Defense of Weed Seeds in Relation to Soil Seedbank Persistence. *Weed Science*, **56**, 676-684. <https://doi.org/10.1614/WS-07-196.1>
- [27] Oigiangbe, N.O. and Onigbinde, A.O. (1996) The Association between Some Physico-Chemical Characteristics and Susceptibility of Cowpea (*Vigna unguiculata* (L.) Walp) to *Callasobruchus maculatus* (F). *Journal of Stored Products Research*, **32**, 7-11. [https://doi.org/10.1016/0022-474X\(96\)00001-X](https://doi.org/10.1016/0022-474X(96)00001-X)
- [28] Mendes-rodrigues, C., Araújo, F.P., Barbosa-Souza, C., Barbosa-Souza, V., Ranal, M.A., Santana, D.G. and Oliveira, P.M. (2010) Multiple Dormancy and Maternal Effect on *Miconia ferruginata* (Melastomataceae) Seed Germination, Serra de Caldas Novas, Goiás, Brazil. *Revista Brasileira de Botânica*, **33**, 93-105. <https://doi.org/10.1590/S0100-84042010000100009>
- [29] Ferro, V.G., Guimarães, P.R. and Trigo, J.R. (2006) Why Do Larvae of *Utetheisa oratrix* Penetrate and Feed in Pods of *Crotalaria* Species? Larval Performance vs. Chemical and Physical Constraints. *Entomologia Experimentalis et Applicata*, **121**, 23-29. <https://doi.org/10.1111/j.1570-8703.2006.00450.x>
- [30] Flores, A.S., Tozzi, A.M.G.A. and Trigo, J.R. (2009) Pyrrolizidine Alkaloid Profiles in *Crotalaria* Species from Brazil: Chemotaxonomic Significance. *Biochemical Systematics and Ecology*, **37**, 459-469. <https://doi.org/10.1016/j.bse.2009.06.001>
- [31] Janzen, D.H. (1970) Herbivores and the Number of Tree Species in Tropical Forests. *American Naturalist*, **104**, 501-527. <https://doi.org/10.1086/282687>

- [32] Vanhoenacker, D., Agren, J. and Éhrlen, J. (2009) Spatial Variability in Seed Predation in *Primula farinosa*: Local Population Legacy versus Patch Selection. *Oecologia*, **160**, 77-86. <https://doi.org/10.1007/s00442-009-1287-z>
- [33] Figueiredo, P.S., Ribeiro, E.K.M.D., Lacerda, D.M.A.L. and Girnos, E.C. (2009) Estratégia reprodutiva de *Cochlospermum orinocense* (Kunth) Steud.: Fenologia, biologia floral e sistema de cruzamento em uma espécie pioneira de florestas na Amazônia. *Revista Brasileira de Botânica*, **32**, 759-770. <https://doi.org/10.1590/S0100-84042009000400016>
- [34] Zar, J.H. (1984) *Biostatistical Analyses*. Prentice Hall, Upper Saddle River.
- [35] Harper, J.L., Lovell, P.H. and Moore, K.G. (1970) The Shapes and Sizes of Seeds. *Annual Review of Ecology and Systematics*, **1**, 327-356. <https://doi.org/10.1146/annurev.es.01.110170.001551>
- [36] Silvertown, J.W. (1989) The Paradox of Seed Size and Adaptation. *Trends in Ecology and Evolution*, **4**, 24-26. [https://doi.org/10.1016/0169-5347\(89\)90013-X](https://doi.org/10.1016/0169-5347(89)90013-X)
- [37] Yanful, M. and Maun, M.A. (1996) Effects of Burial of Seeds and Seedlings from Different Seed Sizes on the Emergence and Growth of *Strophostyleshelvola*. *Canadian Journal of Botany*, **74**, 1322-1330. <https://doi.org/10.1139/b96-160>
- [38] Goodwillie, C., Kalisz, S. and Eckert, C.G. (2005) The Evolutionary Enigma of Mixed Mating Systems in Plants: Occurrence, Theoretical Explanations, and Empirical Evidence. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 47-79. <https://doi.org/10.1146/annurev.ecolsys.36.091704.175539>
- [39] Guyot, S., Vercauteren, J. and Cheyner, V. (1996) Structural Determination of Colourless and Yellow Dimers Resulting from (+)-catechin Coupling Catalysed by Grape Polyphenoloxidase. *Phytochemistry*, **42**, 1279-1288. [https://doi.org/10.1016/0031-9422\(96\)00127-6](https://doi.org/10.1016/0031-9422(96)00127-6)
- [40] Takahama, U. (2004) Oxidation of Vacuolar and Apoplastic Phenolic Substrates by Peroxidase: Physiological Significance of the Oxidation Reactions. *Phytochemistry Review*, **3**, 207-219. <https://doi.org/10.1023/B:PHYT.0000047805.08470.e3>