Which factors influence Demodex proliferation?

A retrospective pilot study highlighting a possible role of subtle immune variations and sebaceous gland status

Running title: Factors influencing Demodex densities

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ABSTRACT

Demodex folliculorum and brevis are commensal mites that live in low densities in human pilosebaceous follicles as part of the normal adult microbiota, but that give rise to demodicosis and, possibly, rosacea, when they proliferate excessively. This proliferation is favored by various factors, including age, marked immunosuppression, sebaceous gland hyperplasia, and hypervascularization-related factors. To study possible factors influencing mite proliferation, we explored the effects of different variables on Demodex densities (Dds) in a retrospective study of two groups of subjects selected on the basis of their clinical diagnosis: Demodex+, consisting of subjects with demodicosis or with centrofacial papulopustules suggesting rosacea (n=844, mean Dd 263.5±8.9 D/cm²), and Demodex-, consisting of subjects with other facial dermatoses or healthy facial skin (n=200, mean Dd 2.3±0.4 D/cm²). Dds were measured using two consecutive standardized skin surface biopsies (SSSB1 [superficial] and SSSB2 [deep]) taken from the same facial site on each subject. In the Demodex+ group: the SSSB1 decreased with age in women (p=0.004), and the SSSB2 increased with age in men (p=0.001) (the pattern was similar for SSSB1+2, but not statistically significant); Dds were lower in those who had received cortisone (either topically or systemically); 13 subjects (1.5%) had known immunosuppression, 62 (7.3%) had hypothyroidism, and in 20 (3.6% of the women) there was a reported link with pregnancy; 78 of the subjects (9.2%) were part of a pair from the same family or household; when associated bacterial infection was suspected, Staphylococcus epidermidis was often isolated. Our results suggest close interactions between the mite, sebaceous gland size and function, and subtle variations of immune status. Potential factors influencing Demodex proliferation...
should be further investigated, including hypothyroidism, pregnancy, corticosteroid administration, *Staphylococcus epidermidis*, contagiousity, and genetic background.

**Key words:** Rosacea; demodicosis; hypothyroidism; sebaceous gland; immunity; cortisone.
INTRODUCTION

Demodex folliculorum and brevis are commensal mites that live in low densities in human pilosebaceous follicles as part of the normal adult microbiota. They are acquired progressively by direct contact with the skin of other humans, mainly within families, and then slowly proliferate over time, under probable control of the host immune system, which they can modulate for their own survival. The Demodex density (Dd) thus increases with age. Many factors have been reported to favor proliferation of the Demodex mite, including immunosuppression, hypervascularization-related factors (likely via vascular endothelial growth factor [VEGF]), which has immunosuppressive properties, lack of use of soap combined with cosmetic overuse, male sex, high concentration of sebaceous glands (such as on the cheeks), sebaceous hyperplasia, and poor blood glucose control. Other factors (e.g., low skin moisture, low sebum quantity, altered sebum composition, and skin alkalinity) may be more a consequence of Demodex proliferation than a cause, because Demodex proliferation probably disturbs sebaceous gland function in the same way it induces Meibomian gland dysfunction on the eyelashes. When the mites proliferate excessively, they become pathogenic, inducing demodicosis and probably, although still controversial, the inflammatory symptoms of rosacea. The concept that the mite is a trigger for rosacea and that high Dds may be a marker of the disease is beginning to be accepted. Recently, using two standardized skin surface biopsies (SSSBs) performed consecutively at the same place on the skin, we confirmed that mean Dds were much higher in subjects with centro-facial papulopustules suggesting rosacea (PPR-
suggestive) and demodicosis than in healthy controls and subjects with other facial
dermatoses. By grouping the PPR-suggestive and demodicosis subjects into a
single Demodex+ group and other subjects into a Demodex- group, we identified
optimal cut-off SSSB (superficial [SSSB1] and deep [SSSB2]) values to differentiate
between “normal” and “abnormal” Dds in these patients. The criterion “a SSSB1 > 5
D/cm² OR a SSSB2 > 10 D/cm²” had a sensitivity of 98.7% and specificity of 95.5%
for a diagnosis of rosacea or demodicosis, providing a valuable diagnostic tool for
dermatologists in routine clinical practice.

In this article, we explored these Demodex+ and Demodex- groups further to
assess factors that may influence Demodex proliferation.

METHODS
This retrospective study was approved by the Erasme Hospital Ethics Committee
and included two groups of patients. All subjects attending our dermatology practice
in Brussels between 2002 and 2010 with a clinical diagnosis of demodicosis (n=590)
or PPR-suggestive (n=254: 215 patients with typical PPR [i.e., centro-facial
papulopustules with persistent erythema] and 39 with other forms [27 centro-facial
papulopustules without persistent erythema, 7 granulomatous rosacea, 5 steroid-
induced rosacea]) were included in a Demodex+ group (n=844). According to our
routine practice, each of these subjects had two consecutive SSSBs performed at the
site of the main skin lesions (preferably on the cheek if affected, because the highest
Dds have been observed here). This procedure enables measurement of
superficial and deep Dds, and calculation of their sum (SSSB1+2), as previously
described. Bacteriological smears were performed only when bacterial infections
were also suspected.
Measurement of Dd is not routine in subjects with other facial dermatoses or in those with healthy facial skin, so such subjects were only included if the consultation time allowed for two SSSBs to be performed (Demodex- group [n=200: 180 with other facial dermatoses and 20 with healthy facial skin]).

For each subject, we recorded their age, sex, and whether or not they regularly used soap on the facial skin. For the Demodex+ subjects, we also recorded topical treatments that had been applied to the facial dermatosis within the past 3 months or longer ago, treatments used for other health problems, general health status (known immunodepression [as a result of illness or treatment] present or existing when the facial dermatosis began, repeated infections, diabetes, thyroid disease), and personal history of atopy. Some subjects spontaneously reported a link between development of their skin condition and pregnancy, which was then recorded. Although we did not routinely look for familial cases, presence of a known case of demodicosis or PPR among the subject’s family or household was recorded.

**Statistical analysis**

Continuous variables are given as means and standard error of the mean (SEM) and qualitative variables as percentages. Differences in the means of continuous variables were compared among groups using Student t-tests in case of two groups. Differences in qualitative variables were compared using exact Pearson chi-square tests. Variation according to age was analyzed separately for men and women using linear regression models. Statistical significance was considered for \( p < 0.05 \). All statistical tests were performed using IBM-SPSS (version 22.0 to 27.0) software (IBM Corp, Armonk, NY, USA).
RESULTS

Among the 1044 subjects, the mean age was 44.6±0.5 years (range: 7.4–98.3); 712 (68.2%) were women. Subjects in the Demodex- group (mean SSSB1+2: 2.3±0.4 D/cm²) were younger (36.5±0.9 vs. 46.5±0.5 years, p<0.001) and more likely to be female (150/200 [75.0%] vs.562/844 [66.6%], p=0.023) than subjects in the Demodex+ group (mean SSSB1+2: 263.5±8.9 D/cm²). From about 40 years of age, the relative proportion of Demodex+ subjects increased with age (Fig. 1). In the Demodex- group, women were older than men (38.1±1.1 vs. 31.9±1.8 years, p=0.005) and their SSSB2 and SSSB1+2 increased with age (Fig. 2). Among the subjects for whom data on soap use were available, the proportion that regularly used soap was similar in Demodex+ and Demodex- subjects (349/833 [41.9%] vs 62/149 [41.6%], p=0.948).

Demodex+ Group

1. Age and sex

In the Demodex+ group, the mean age was not significantly different in men and women. Dds varied according to sex, with the mean SSSB2 and SSSB1+2 higher in men than in women (Table 1). The SSSB1 decreased with age in women (p=0.004), and the SSSB2 increased with age in men (p=0.001); the same tendency was present for SSSB1+2 (decreasing in women: p=0.069; increasing in men: p=0.062) (Fig. 2).

2. SSSB site and use of soap

SSSB2 and SSSB1+2 values were lower on the nose than elsewhere (Table 1).
There were no statistically significant differences in mean Dds according to whether or not soap was used (respective p values for SSSB1, SSSB2 and SSSB1+2, p=0.710, 0.771 and 0.944).

3. General health status and pregnancy

Only 13 (1.5%) subjects were known to be immunosuppressed: 5 with immunosuppressive disease (leukemia receiving cortisone [n=1], leukopenia [n=1], neutrophil polymorphonuclear chemotactic deficiency [n=1], sarcoidosis [n=1], alcoholic hepatitis [n=1]) and 8 who were receiving immunosuppressive treatment (corticotherapy [n=3], chemotherapy [n=2], oral tacrolimus [n=1], radioactive iodine [n=1], monoclonal antibodies [n=1]). One subject was pregnant, 4 had a history of recurrent infections, 16 of diabetes, and 76 of thyroid disease (62 hypothyroid, 1 hyperthyroid, 11 euthyroid and 2 unknown); 234 had a personal history of atopy.

Twenty of the 562 women (3.6%) reported a link with pregnancy: 15 said the skin condition appeared during pregnancy (Fig. 3a,b) and 5 that it worsened during or just after pregnancy.

4. Medication

a. Prior treatments for demodicosis or rosacea

Among the 831 subjects (98%) for whom the information was available, 372 (45%) had received prior treatment for their skin condition: acaricidal treatments (n=85), metronidazole (69 topical, 6 oral), oral tetracyclines (n=72), topical cortisone (n=69), topical antibiotic (n=57), anti-mycotic treatment (oral or topical, n=30), oral isotretinoin (n=21), topical anti-mycotic+corticoid (n=19), topical retinoids (n=10), vascular lasers (n=10), clonidine (n=5), peeling treatments (n=4), antiseptic solutions (n=4), azelaic acid (n=2), and other treatments (n=37); some subjects received more than one treatment. There were no statistically significant differences in the mean
Dds in subjects who had received recent treatment (n=209), less recent treatment (n=163), or no treatment (n=459).

Among the 163 subjects who had only received treatment >3 months previously, there were no statistically significant differences in the mean Dds across the different treatments. In the 209 subjects who had received more recent treatment, only subjects who had received an acaricidal agent (n=28) or a topical “anti-mycotic+corticoid” (n=10) treatment had lower SSSB2 and SSSB1+2 values than those who had received another recent treatment (Table 1). In a subgroup analysis in subjects who had not recently received an oral/systemic immunosuppressive agent or cortisone (for their facial dermatosis or other condition, n=816), the 7 subjects with recent topical “anti-mycotic+corticoid” only (i.e., no other recent treatment) had a lower SSSB1+2 than the 601 without any recent treatment (122±33 vs 268±10 D/cm², p=0.004). The same tendency was observed for the 15 patients with recent acaricidal treatment only (171±46 vs 268±10 D/cm², p=0.140).

b. Medication for other conditions, and the contraceptive pill

Among the 832 subjects for whom the information was available, 467 (56.1%) were taking at least one medication for conditions other than their skin disease, including 231 women taking the contraceptive pill. There were no statistically significant differences in the mean Dds in the 139 women taking only a contraceptive pill and the 191 women taking no medication (Table 1). The 325 subjects who were taking at least one medication other than the contraceptive pill were older than the 360 who were not (54.3±0.9 vs 42.2±0.7 years; p<0.001), but Dds were not significantly different (Table 1).
c. **Cortisone** *(for facial dermatosis or other conditions)*

A total of 87 (10.3%) subjects had been recently exposed to cortisone, either topically (n=63) or orally/by inhalation (n=24). The mean SSSB1 and SSSB1+2 in these subjects were significantly lower than those in the 757 subjects not exposed to cortisone (Table 1). The mean Dds tended to be lower in our 5 subjects with steroid-induced rosacea (Fig. 3c) than in our 215 subjects with typical PPR (Table 1).

5. **Bacterial infections**

Bacteriological smears were performed in 19 subjects; culture results showed *Staphylococcus aureus* (n=1), *Staphylococcus epidermidis* (n=11), Gram-positive cocci but no pathogenic bacteria (n=5), and no bacteria detected (n=2) (Fig. 3d).

6. **Familial cases**

Seventy-eight of the subjects had another family or household member who also consulted, forming 39 pairs: in nine of the pairs, the subjects had no genetic link but were living together, and in 30 pairs, the subjects were genetically related (25 “parent/child” and 5 “brother/sister”) (Fig. 3e,f).

**DISCUSSION**

The main findings of our study were that, in the Demodex+ patients (with high Dds), female sex and cortisone exposure were associated with lower Dd values, there was a tendency for the high Dds to decrease with age in women and to increase with age in men, and there was a high prevalence of hypothyroidism.

1. **Age and sex**

Dds increased with age in our subjects as reported previously.\(^3,13–17\) Demodex proliferation is probably favored by the development of sebaceous glands during
puberty\(^3\) and later by the slow decrease with age of T-cell mediated immunity,\(^{46}\) which is implicated in the response to mite proliferation.\(^9,20,42,47–49\)

In the Demodex- group, there were no significant difference in Dds in men and women, which may be expected because of the low densities overall in that group. Nevertheless, there was a small increase in Dd with age in women, which may be explained by the presence of more subjects with subclinical demodicosis in older age groups,\(^{25}\) as an indirect consequence of increasing proliferation of Demodex with age.

In the Demodex+ group, the higher mean total Dd in men than in women was particularly evident among older subjects because the Dd tended to decrease with age in women and to increase in men. These observations are compatible with most previous studies\(^3,13–17\) and with the plateau effect observed from age 60-65 years in the general population:\(^14,50\) after a certain age, the decrease in Dd in women may counterbalance the increase in men, thus creating an apparent plateau. The differences in Dd with age and sex may be explained by the influence on Demodex proliferation of the size and density of sebaceous glands/pilosebaceous follicles, which are regulated by androgens which in turn depend on age and sex.\(^51\)

The mites are dependent on the pilosebaceous follicles for habitat and nutrition,\(^1,10,47,52,53\) and it is here that immunotolerance is observed.\(^54\) These factors explain why the mites are only found in skin areas rich in sebaceous glands\(^7,53\) and why their number increases when sebaceous gland density increases.\(^16\)

The influence of androgens on sebaceous glands explains why glands are larger in men,\(^55\) and why pilosebaceous follicles involute with age,\(^46,50,56,57\) an effect that occurs 10 to 20 years earlier in women than in men.\(^46,56,57\) Gland involution in older age is therefore probably responsible for a mite saturation effect, occurring
earlier in women. This indirect influence of androgens on Dd, may also help explain why a higher Dd has been observed in subjects with polycystic ovary syndrome, \(^{58}\) why subjects with acne may be infested, \(^{6,31,59}\) why \(D. \text{brevis}\) prevalence may correlate with acne severity, \(^{31}\) and why demodicosis and rosacea are frequently present concurrently with seborrheic dermatitis \(^{27}\) or acne vulgaris. \(^{3,24,27,31,37,38,45,50,59,60}\)

### 2. SSSB site and use of soap

Although the age and sex effects suggest the importance of sebaceous gland size and density on Dds, our study paradoxically also showed that Dds on the nose (with large sebaceous glands) although high, were lower than elsewhere on the face, as also reported in other studies. \(^{4,13,32,33,53}\) A possible explanation for this finding could be that these large glands may mostly favor the mites that live deep inside sebaceous glands (\(D. \text{brevis}\) and a short-bodied transparent form of \(D. \text{folliculorum}\)), \(^{61}\) thus, not effectively detected by SSSB. \(^{62}\) Indeed, one histological study suggested a higher proportion of \(D. \text{brevis}\) to \(D. \text{folliculorum}\) (long-bodied phenotypes) on the nose than on other facial areas tested. \(^{53}\) The deeper mites may compete with the more superficial ones, thus limiting their proliferation. Alternatively, do the pilosebaceous follicles of the nose have other characteristics (e.g., shorter infundibula) that could limit the proliferation of superficial \(D. \text{folliculorum}\)?

In 1932, Ayres suspected that not washing the face with soap, combined with excessive use of cosmetic creams, could favor abnormal mite proliferation. \(^{30}\) The influence of hygiene has been confirmed in other studies, \(^{50,63}\) but in our study, use of soap did not seem to influence the Dd \(^{3,37}\) likely because our subjects' hygiene was generally good even if they did not use soap.
3. General health & pregnancy

Although immunodepression of various origins\textsuperscript{18–23,64} (including use of corticoids\textsuperscript{4,34,50,65}) may promote Demodex proliferation, demodicosis and rosacea generally occur in subjects who are otherwise apparently in good health.\textsuperscript{5,30,37,66}

Among our Demodex+ subjects, marked immunodepression was rare. Diabetes and a history of atopy were present in similar frequencies to those reported in the general population,\textsuperscript{67,68} although the prevalence of hypothyroidism (7.3\%) was almost twice that of all combined thyroid disorders in Brussels (about 4\%) during the same period as our study.\textsuperscript{69} Among the limited epidemiological data available, it is interesting to note that the incidence ratio of hypo-:hyperthyroidism was 2:1 in Brussels (during 1996 and 1997),\textsuperscript{70} whereas the prevalence ratio was 62:1 in our patients. These observations, although limited by the nature of the study, suggest a potential role of hypothyroidism in favoring Demodex proliferation (which is well-known in veterinary medicine)\textsuperscript{71} perhaps induced by associated decreased immune response.\textsuperscript{72}

Interestingly, in mice T-cell immunosuppression related to hypothyroidism was not improved by thyroid hormone therapy alone but required additional zinc supplementation.\textsuperscript{73}

Only one subject was pregnant, but 20 spontaneously reported the onset or worsening of their demodicosis/PPR during their pregnancy, suggesting that pregnancy-related factors, for example the associated relative immunosuppression, may favor Demodex proliferation, although Aydingöz \textit{et al.} reported no significant difference in Dds in pregnant and non-pregnant women.\textsuperscript{74}

Thus, in addition to severe immunodepression, subtle immune defects (as seen in pregnancy, in hypothyroidism, and with age, not forgetting the likely
immunosuppressive action of VEGF\textsuperscript{12,29} associated with ETR\textsuperscript{75}), which are more frequently encountered in daily practice, can also favor Demodex proliferation.

4. Medication

Among the recent treatments used for the skin condition, only two seemed to be associated with relatively lower Dds: topical acaricidal treatment and the “anti-mycotic+corticoid” combination. Subjects recently exposed to any cortisone (topical or systemic) had lower Dds than those not exposed. However, Dds were similar whether or not subjects had recently been exposed to an anti-mycotic (topical or systemic), suggesting that the relatively lower Dds observed among subjects with recent “anti-mycotic+corticoid” treatment was related to the action of cortisone and not to that of the anti-mycotic. Nevertheless, cortisone is usually associated with a favorable effect on Demodex proliferation,\textsuperscript{4,34,50,65} suggesting that cortisone may have two opposite actions on Demodex proliferation: favoring its proliferation when Dd is low (via an immunosuppressive effect\textsuperscript{76,77}), and thus being able to induce steroid-induced rosacea, but limiting proliferation when Dd is high, perhaps because of an antiproliferative effect on sebaceous cells/pilosebaceous follicles.\textsuperscript{3,78} This hypothesis is in accordance with a study by Zhao \textit{et al}, in which Demodex infestation was associated with steroid-induced dermatitis but in which high Dds tended to be less frequent in subjects with steroid-induced dermatitis than in those with other types of rosacea (19.2% vs 38.5%).\textsuperscript{50} Dds also tended to be lower in our patients with steroid-induced rosacea than in PPR, although the small number of such subjects in our cohort does not allow us to draw firm conclusions.

By contrast with the findings of Zeytun,\textsuperscript{14} our subjects taking medication, although older than those who were not, did not have higher Dds.
5. Bacterial infection

*S. epidermidis* was identified fairly frequently, compatible with other studies showing its frequent detection in patients with rosacea (from 28% to 60% of subjects) and supports its probable, occasional interaction with the mite. Indeed, via its lipoteichoic acid (which exerts an anti-inflammatory action on keratinocytes), it may favor its own proliferation but also that of the Demodex mite. On the other hand, the abrasive action of the Demodex mite may favor contact of the lipoteichoic acid with the dermis, enhancing the proinflammatory action of lipoteichoic acid on immune cells that normally exist in a sterile environment.

Two subjects with deep folliculitis had no detectable pathogenic bacteria suggesting that Demodex itself may also induce these symptoms, which could then be considered as a variant of a demodectic isolated inflammatory papule.

6. Familial cases

Genetic factors are well-known to influence the risk of rosacea, and the STAT 1 gain-of-function mutation is associated with familial rosacea and demodicosis. In our study, most of the pairs of subjects had a genetic link suggesting that genetic background is more important than just close contact with a subject with high Dd. Nevertheless, the fact that individuals with no genetic link but from the same household were also affected suggests that frequent contact with a person with high Dd may lead to transfer of numerous mites, indicating possible contagion.

7. Limitations, conclusions and perspectives

Our study has several limitations including its retrospective nature, the non-systematic inclusion of subjects with healthy skin or with other facial dermatosis, the relatively small number of subjects with healthy facial skin, and the lack of data on
other health problems in our Demodex- subjects and on severity and treatment of hypothyroidism in our Demodex+ subjects.

Nevertheless, our results suggest that close interactions may exist between the Demodex mite, sebaceous gland size and function, and subtle variations in immunity, including hypothyroidism (Fig. 4).

The potential influence of hypothyroidism, pregnancy, corticosteroid administration, bacterial interactions, contagiosity and genetic background on Demodex proliferation should be further investigated.

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Authors declare no Conflict of Interests for this article
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Figure legends

Figure 1. Proportion of subjects in Demodex- and Demodex+ groups according to age

The known increase in Demodex density with age\textsuperscript{3,13–17} may explain why our Demodex- (gray columns) were generally younger than our Demodex+ (orange columns) subjects. Indeed, many older subjects attending our practice with apparently healthy skin were, under careful examination, found to have telangiectasia and/or follicular scales suggesting demodicosis. This suggests that in most elderly subjects, the skin is probably not healthy.
Figure 2. Variation in Demodex densities (Dds) with age in men and women in Demodex- (panels a, b and c) and Demodex+ (panels d, e and f) groups.

(a, b, c): Among Demodex- subjects (n=200), SSSB2 and SSSB1+2 increase with age in women (p=0.002 and p=0.006 respectively). In older women there was considerable variation in the measured densities: some subjects diagnosed clinically as “healthy” or as “other facial dermatoses” nevertheless had high Dd values. The higher SSSB2 values in subjects with a normal SSSB1, reflects Demodex proliferation deeper in the follicles: this may help explain why the proliferation was not clinically visible. The increase in Dd with age observed among women was not observed among men, but we only had one male Demodex- subject older than 55 years.

(d, e, f): Among Demodex+ subjects (n=844), SSSB1 decreases with age in women (p=0.004) and SSSB2 increases with age in men (p=0.001). SSSB2 and SSSB1+2 were higher in men than in women, particularly in older subjects.
Figure 3. Rosacea and demodicosis: Clinical cases

(a) and (b): A 52-year-old woman with rosacea with papulopustules (PPR) who spontaneously told us that the PPR began after her first pregnancy 31 years previously. The follicular scales and pustules are clearly visible.

(c): 73-year-old man with steroid-induced rosacea secondary to topical fluticasone-17-propionate 0.5/1000 used for 2-3 months for PPR persisting for 12 years.

(d): Deep folliculitis observed for 1 week on the cheek of a 43-year-old woman, without fever. Clinical examination revealed pityriasis folliculorum. A bacterial smear was negative.

(e) and (f): A 48-year-old woman presented with rosacea with papulopustules (PPR) present for 5 years (e); 3 months after her first consultation, her 18-year-old daughter consulted for a less severe PPR present for 2 years with associated acne and seborrheic dermatitis (f). SSSB1 +SSSB2 values are indicated on the figures.

These five patients were included in the study and provided written consent for publication.
Figure 4. Hypothesis assembling some of the main factors that may influence Demodex proliferation: this highlights the close interactions existing between Demodex mite, sebaceous gland size and function, and the immune system.

Age and sex influence Demodex proliferation likely via the production of androgens and their effect on sebaceous glands, where the mites live. Immune defects (ID) of various origins seem to be another major factor favoring Demodex proliferation: marked immunodepression (rare, e.g., associated with HIV [human immunodeficiency virus] infection), and, more frequently, more subtle IDs, e.g., associated with thymic stromal lymphopoietin (TSLP, increased in sebaceous gland rich areas), glucocorticoids (of which abnormal endogenous synthesis is encountered in rosacea), vascular endothelial growth factor (VEGF, which is increased in rosacea and may induce T-cell exhaustion as in tumor pathology), Staphylococcus epidermidis, and, potentially, pregnancy and hypothyroidism.

Corticosteroids may favor proliferation when Demodex densities (Dds) are low, via an immunosuppressive effect, and could limit its excessive proliferation when Dds are high, by an atrophic action on the pilosebaceous follicles. Good facial hygiene may also potentially reduce mite proliferation and some topical treatments are clearly acaricidal (ivermectin, benzyl benzoate).

Genetic influence may play a role at different levels (black asterisks), including as a potential specific ID limited to the defense against the mite. Finally, Demodex mites themselves likely also control the immune system of the host for their own benefit.
## Table 1. Demodex densities (Dds) according to different variables

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<th>SSSSB 1</th>
<th>SSSSB2</th>
<th>SSSSB1+2</th>
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<td>n % Mean ± SEM</td>
<td>Min - Max</td>
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<td><strong>DEMODEX- GROUP</strong></td>
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<td>Women</td>
<td>150 75</td>
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<td><strong>DEMODEX+ GROUP</strong></td>
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<td>82 ± 5 0 - 756</td>
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<td>Men</td>
<td>282 33</td>
<td>88 ± 6 0 - 636</td>
<td>0.001</td>
</tr>
<tr>
<td>All</td>
<td>844 100</td>
<td>84 ± 4 0 - 756</td>
<td>-</td>
</tr>
<tr>
<td><strong>Biopsy site</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheek</td>
<td>710 84.1</td>
<td>86 ± 4 0 - 756</td>
<td>0.001</td>
</tr>
<tr>
<td>Chin</td>
<td>62 7.3</td>
<td>74 ± 13 0 - 408</td>
<td>0.001</td>
</tr>
<tr>
<td>Forehead</td>
<td>37 4.4</td>
<td>86 ± 18 0 - 520</td>
<td>0.001</td>
</tr>
<tr>
<td>Temple</td>
<td>19 2.3</td>
<td>79 ± 15 1 - 224</td>
<td>0.001</td>
</tr>
<tr>
<td>Nose</td>
<td>9 1.1</td>
<td>8 ± 3 0 - 25</td>
<td>0.001</td>
</tr>
<tr>
<td>Other</td>
<td>7 0.8</td>
<td>53 ± 18 7 - 149</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Steroid-induced rosacea</strong></td>
<td>5 0.6</td>
<td>42 ± 37 0 - 188</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Typical PPR</strong></td>
<td>215</td>
<td>91 ± 8 0 - 756</td>
<td>-</td>
</tr>
</tbody>
</table>

### Note

- **a**: The Demodex- group included 20 subjects with clinically healthy facial skin and 180 with diverse facial dermatoses.
- **b**: The Demodex+ group included 590 subjects with demodicosis and 254 with centrofacial papulopustules suggesting rosacea (215 with persistent erythema, 27 without persistent erythema, 7 granulomatous rosacea and 5 steroid-induced rosacea).
- **c**: The 710 cheeks contained 6 nasolabial folds.
- **d**: Mean SSSSB2 and SSSSB1+2 values were lower on the nose than elsewhere: vs cheek (p<0.001 both), chin (p=0.001 and p<0.001), forehead (p=0.003 and p<0.001) and temple (p=0.022 and p=0.001).
- **e**: The 7 other locations were: 2 preauricular area, 2 inferior eyelids, 1 white part of the upper lip, 1 vertex, and 1 neck.
- **f**: Steroid-induced rosacea: diagnosis considered when a patient with central papulopustules suggesting a diagnosis of rosacea had received significant amounts of topical or oral corticosteroids over a prolonged period of time.
- **g**: Typical PPR: centro-facial papulopustules with persistent erythema.
- **h**: Sixty-three subjects had been recently exposed to topical cortisone (53 cortisone alone, 9 cortisone associated with antifungal, one subject with both) and 24 subjects had been recently exposed to cortisone orally or by inhalation.
- **i**: The medications were: anti-hypertensive drugs (n=95), thyroid drugs (n=61), anti-depressives (n=34), anxiolytics (n=31), cortisone (orally or by inhalation: n=24), gastric agents (n=21), immunosuppressives (n=8), and others or not specified (n=222).
Figure 1. Proportion of subjects in Demodex- and Demodex+ groups according to age. The known increase in Demodex density with age\textsuperscript{3,13–17} may explain why our Demodex- (gray columns) were generally younger than our Demodex+ (orange columns) subjects. Indeed, many older subjects attending our practice with apparently healthy skin were, under careful examination, found to have telangiectasia and/or follicular scales suggesting demodicosis. This suggests that in most elderly subjects, the skin is probably not healthy.
Figure 2. Variation in Demodex densities (Dds) with age in men and women in Demodex- (panels a, b and c) and Demodex+ (panels d, e and f) groups.

(a, b, c): Among Demodex- subjects (n=200), SSSB2 and SSSB1+2 increase with age in women (p=0.002 and p=0.006 respectively). In older women there was considerable variation in the measured densities: some subjects diagnosed clinically as “healthy” or as “other facial dermatoses” nevertheless had high Dd values. The higher SSSB2 values in subjects with a normal SSSB1, reflects Demodex proliferation deeper in the follicles: this may help explain why the proliferation was not clinically visible. The increase in Dd with age observed among women was not observed among men, but we only had one male Demodex- subject older than 55 years.

(d, e, f): Among Demodex+ subjects (n=844), SSSB1 decreases with age in women (p=0.004) and SSSB2 increases with age in men (p=0.001). SSSB2 and SSSB1+2 were higher in men than in women, particularly in older subjects.
Figure 3. Rosacea and demodicosis: Clinical cases

(a) and (b): A 52-year-old woman with rosacea with papulopustules (PPR) who spontaneously told us that the PPR began after her first pregnancy 31 years previously. The follicular scales and pustules are clearly visible.

(c): 73-year-old man with steroid-induced rosacea secondary to topical fluticasone-17-propionate 0.5/1000 used for 2-3 months for PPR persisting for 12 years.

(d): Deep folliculitis observed for 1 week on the cheek of a 43-year-old woman, without fever. Clinical examination revealed pityriasis folliculorum. A bacterial smear was negative.

(e) and (f): A 48-year-old woman presented with rosacea with papulopustules (PPR) present for 5 years (e); 3 months after her first consultation, her 18-year-old daughter consulted for a less severe PPR present for 2 years with associated acne and seborrheic dermatitis (f). SSSB1 + SSSB2 values are indicated on the figures.

These five patients were included in the study and provided written consent for publication.
Figure 4. Hypothesis assembling some of the main factors that may influence Demodex proliferation: this highlights the close interactions existing between Demodex mite, sebaceous gland size and function, and the immune system.

Age and sex influence Demodex proliferation likely via the production of androgens and their effect on sebaceous glands, where the mites live. Immune defects (ID) of various origins seem to be another major factor favoring Demodex proliferation: marked immunodepression (rare, e.g., associated with HIV [human immunodeficiency virus] infection), and, more frequently, more subtle IDs, e.g., associated with thymic stromal lymphopoietin (TSLP, increased in sebaceous gland rich areas), glucocorticoids (of which abnormal endogenous synthesis is encountered in rosacea), vascular endothelial growth factor (VEGF, which is increased in rosacea and may induce T-cell exhaustion as in tumor pathology), Staphylococcus epidermidis, and, potentially, pregnancy and hypothyroidism. Corticosteroids may favor proliferation when Demodex densities (Dds) are low, via an immunosuppressive effect, and could limit its excessive proliferation when Dds are high, by an atrophic action on the pilosebaceous follicles. Good facial hygiene may also potentially reduce mite proliferation, and some topical treatments are clearly acaricidal (ivermectin, benzyl benzoate).

Genetic influence may play a role at different levels (black asterisks), including as a potential specific ID limited to the defense against the mite.

Finally, Demodex mites themselves likely also control the immune system of the host for their own benefit.
<table>
<thead>
<tr>
<th>Biopsy site</th>
<th>n</th>
<th>%</th>
<th>Mean ± SEM</th>
<th>Min - Max</th>
<th>p</th>
<th>Mean ± SEM</th>
<th>Min - Max</th>
<th>p</th>
<th>Mean ± SEM</th>
<th>Min - Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheek</td>
<td>710</td>
<td>84.1</td>
<td>86 ± 4</td>
<td>0 - 756</td>
<td>0.457</td>
<td>188 ± 7</td>
<td>0 - 1280</td>
<td>0.302</td>
<td>141 ± 58</td>
<td>7 - 149</td>
<td>0.021</td>
</tr>
<tr>
<td>Chin</td>
<td>62</td>
<td>7.3</td>
<td>74 ± 13</td>
<td>0 - 408</td>
<td>0.457</td>
<td>132 ± 22</td>
<td>1 - 860</td>
<td>0.025</td>
<td>213 ± 35</td>
<td>21 - 488</td>
<td>0.001</td>
</tr>
<tr>
<td>Forehead</td>
<td>37</td>
<td>4.4</td>
<td>86 ± 18</td>
<td>0 - 520</td>
<td>0.010</td>
<td>160 ± 31</td>
<td>2 - 1020</td>
<td>0.025</td>
<td>246 ± 40</td>
<td>13 - 1060</td>
<td>0.012</td>
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<td>Temple</td>
<td>19</td>
<td>2.3</td>
<td>79 ± 15</td>
<td>1 - 224</td>
<td>0.021</td>
<td>135 ± 28</td>
<td>11 - 376</td>
<td>0.001</td>
<td>214 ± 35</td>
<td>21 - 488</td>
<td>0.017</td>
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<tr>
<td>Nose</td>
<td>9</td>
<td>1.1</td>
<td>8 ± 3</td>
<td>0 - 25</td>
<td>0.021</td>
<td>27 ± 9</td>
<td>3 - 82</td>
<td>0.021</td>
<td>34 ± 12</td>
<td>5 - 107</td>
<td>0.021</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>0.8</td>
<td>53 ± 18</td>
<td>7 - 149</td>
<td>0.021</td>
<td>42 ± 7</td>
<td>0 - 108</td>
<td>0.021</td>
<td>20 ± 14</td>
<td>1 - 1280</td>
<td>0.021</td>
</tr>
<tr>
<td>Steroid-induced rosacea</td>
<td>5</td>
<td>42 ± 37</td>
<td>0 - 188</td>
<td>0.057</td>
<td>157 ± 88</td>
<td>0 - 440</td>
<td>0.057</td>
<td>200 ± 108</td>
<td>0 - 476</td>
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<tr>
<td>Typical PPR</td>
<td>215</td>
<td>91 ± 8</td>
<td>0 - 756</td>
<td>0.021</td>
<td>208 ± 14</td>
<td>1 - 1280</td>
<td>0.021</td>
<td>298 ± 19</td>
<td>5 - 1464</td>
<td>0.021</td>
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</tbody>
</table>

### Some of the topical treatments among subjects with recent treatment (n=209)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>%</th>
<th>Mean ± SEM</th>
<th>Min - Max</th>
<th>p</th>
<th>Mean ± SEM</th>
<th>Min - Max</th>
<th>p</th>
<th>Mean ± SEM</th>
<th>Min - Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acaricidal</td>
<td>28</td>
<td>13.4</td>
<td>69 ± 13</td>
<td>0 - 135</td>
<td>0.021</td>
<td>118 ± 24</td>
<td>0 - 185</td>
<td>0.021</td>
<td>186 ± 34</td>
<td>0 - 135</td>
<td>0.021</td>
</tr>
<tr>
<td>Other</td>
<td>181</td>
<td>86.6</td>
<td>86 ± 8</td>
<td>0 - 135</td>
<td>0.021</td>
<td>185 ± 15</td>
<td>0 - 185</td>
<td>0.021</td>
<td>271 ± 21</td>
<td>0 - 185</td>
<td>0.021</td>
</tr>
<tr>
<td>Antimycotic alone</td>
<td>21</td>
<td>10.0</td>
<td>96 ± 28</td>
<td>0 - 135</td>
<td>0.021</td>
<td>184 ± 33</td>
<td>0 - 185</td>
<td>0.021</td>
<td>281 ± 54</td>
<td>0 - 135</td>
<td>0.021</td>
</tr>
<tr>
<td>Other</td>
<td>188</td>
<td>90.0</td>
<td>82 ± 7</td>
<td>0 - 135</td>
<td>0.021</td>
<td>175 ± 15</td>
<td>0 - 185</td>
<td>0.021</td>
<td>257 ± 20</td>
<td>0 - 135</td>
<td>0.021</td>
</tr>
<tr>
<td>Cortisone alone</td>
<td>54</td>
<td>25.8</td>
<td>66 ± 12</td>
<td>0 - 135</td>
<td>0.021</td>
<td>144 ± 22</td>
<td>0 - 185</td>
<td>0.021</td>
<td>211 ± 31</td>
<td>0 - 135</td>
<td>0.021</td>
</tr>
<tr>
<td>Other</td>
<td>155</td>
<td>74.2</td>
<td>90 ± 9</td>
<td>0 - 135</td>
<td>0.021</td>
<td>187 ± 17</td>
<td>0 - 185</td>
<td>0.021</td>
<td>276 ± 23</td>
<td>0 - 135</td>
<td>0.021</td>
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<tr>
<td>Cortisone + antimycotic</td>
<td>10</td>
<td>4.8</td>
<td>68 ± 13</td>
<td>0 - 135</td>
<td>0.021</td>
<td>67 ± 21</td>
<td>0 - 185</td>
<td>0.021</td>
<td>135 ± 24</td>
<td>0 - 135</td>
<td>0.021</td>
</tr>
<tr>
<td>Other</td>
<td>199</td>
<td>95.2</td>
<td>85 ± 8</td>
<td>0 - 135</td>
<td>0.021</td>
<td>181 ± 14</td>
<td>0 - 185</td>
<td>0.021</td>
<td>266 ± 19</td>
<td>0 - 135</td>
<td>0.021</td>
</tr>
</tbody>
</table>

### Exposed to topical or systemic cortisone

- **DEMODEX- GROUP**
  - Women:
    - 150 participants, 75% with 1 ± 0 (min - max: 0 - 23, p = 0.775)
    - 2 ± 0 (min - max: 0 - 35, p = 0.712)
  - Men:
    - 50 participants, 25% with 1 ± 0 (min - max: 0 - 6, p = 0.775)
    - 2 ± 0 (min - max: 0 - 27, p = 0.712)
  - All:
    - 200 participants, 100% with 1 ± 0 (min - max: 0 - 23, p = 0.775)
    - 2 ± 0 (min - max: 0 - 35, p = 0.712)

- **DEMODEX+ GROUP**
  - Women:
    - 562 participants, 67% with 167 ± 8 (min - max: 0 - 1256, p = 0.010)
    - 249 ± 10 (min - max: 0 - 1456, p = 0.024)
  - Men:
    - 282 participants, 33% with 204 ± 12 (min - max: 0 - 1280, p = 0.025)
    - 292 ± 16 (min - max: 11 - 1464, p = 0.017)
  - All:
    - 844 participants, 100% with 188 ± 7 (min - max: 0 - 1280, p = 0.021)
    - 264 ± 9 (min - max: 0 - 1464, p = 0.017)

### Biopsy site
- **Cheek**:
  - 150 participants, 75% with 1 ± 0 (min - max: 0 - 23, p = 0.775)
  - Mean ± SEM: 0.0 - 23
  - Other:
    - 10 participants, 5% with 0 ± 0 (min - max: 0 - 0, p = 0.775)
    - Mean ± SEM: 0.0 - 0

### Steroid-induced rosacea
- **Typical PPR**:
  - 5 participants, 2.5% with 42 ± 37 (min - max: 0 - 188, p = 0.371)
  - Other:
    - 181 participants, 90.5% with 86 ± 13 (min - max: 0 - 135, p = 0.021)
    - Other:
      - 28 participants, 14% with 69 ± 13 (min - max: 0 - 135, p = 0.021)

### Some of the topical treatments among subjects with recent treatment (n=209)

- **Acaricidal**:
  - 28 participants, 10% with 69 ± 13 (min - max: 0 - 135, p = 0.021)
  - Other:
    - 181 participants, 90% with 86 ± 13 (min - max: 0 - 135, p = 0.021)

- **Antimycotic alone**:
  - 21 participants, 7% with 96 ± 28 (min - max: 0 - 135, p = 0.021)

- **Cortisone alone**:
  - 54 participants, 20% with 66 ± 12 (min - max: 0 - 135, p = 0.021)

- **Cortisone + antimycotic**:
  - 10 participants, 4% with 68 ± 13 (min - max: 0 - 135, p = 0.021)

- **Exposed to topical or systemic cortisone**:
  - 209 participants, 100% with 86 ± 13 (min - max: 0 - 135, p = 0.021)
<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>65 ± 9</th>
<th>86 ± 4</th>
<th>144 ± 18</th>
<th>184 ± 7</th>
<th>209 ± 25</th>
<th>270 ± 10</th>
<th>0.026</th>
<th>0.061</th>
<th>0.036</th>
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<tbody>
<tr>
<td><strong>Contraceptive pill (among women)</strong></td>
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<td></td>
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<tr>
<td>Contraceptive pill alone</td>
<td>139</td>
<td>16.5</td>
<td>94 ± 10</td>
<td>184 ± 16</td>
<td>0.332</td>
<td>278 ± 23</td>
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<tr>
<td>No medication</td>
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<td>22.6</td>
<td>82 ± 8</td>
<td>172 ± 13</td>
<td>0.574</td>
<td>255 ± 18</td>
<td>0.419</td>
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<tr>
<td><strong>Medication (other than contraceptive pill)</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>325</td>
<td>80 ± 6</td>
<td>183 ± 11</td>
<td>180 ± 9</td>
<td>0.432</td>
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<tr>
<td>No medication</td>
<td>360</td>
<td>86 ± 6</td>
<td>180 ± 9</td>
<td>267 ± 13</td>
<td>0.838</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

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\[h\]: Sixty-three subjects had been recently exposed to topical cortisone (53 cortisone alone, 9 cortisone associated with antifungal, one subject with both) and 24 subjects had been recently exposed to cortisone orally or by inhalation.  

\[i\]: The medications were: anti-hypertensive drugs (n=95), thyroid drugs (n=61), anti-depressives (n=34), anxiolytics (n=31), cortisone (orally or by inhalation; n=24), gastric agents (n=21), immunosuppressives (n=4), and others or not specified (n=222).