The Mirror Phenomenon in Monozygotic Twins

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38

BACKGROUND

For years it was commonly believed that approximately 25% of monozygotic (MZ) twins exhibited what was loosely referred to as the 'mirror phenomenon'. In this situation, what was seen anatomically on the left side of the body in one twin was seen on the right side of the other twin. Mirror-image features in twins commonly include moles, birthmarks, tooth eruption patterns and hair whorls. According to some authors, the mirror phenomenon is also a partial explanation for why more than a third of MZ twins are left-handed, double the rate in the general population. In contrast, only rarely are the internal organs reversed in one of the twins (situs inversus)[1].

The current explanation for this phenomenon relates to zygotic splitting at a relatively advanced stage, usually at day 7 or beyond[1,2]. In earlier zygotic splitting, twins are formed by the separation of the zygote into two cells or two small clusters of cells that are derived from one fertilized egg. To the best of our knowledge, there is no three-dimensional orientation to these cells or tiny cell clusters. In later splits, however, it is considered logical that the developing cell mass has already attained some degree of polarity, that is, a right and a left side. In this construct, following division of the cell mass, both sides are like the mirror reflection of each other, rather than an exact duplicate[1]. This construct may be flawed, however, as it presumes that the split always favors laterality rather than a transverse or arbitrary separation. Because no three-dimensional observation of the splitting process has been made *in vivo*, and because polarity is not visible to the observer in *in vitro* culture preparations, the details of this process remain speculative.

Although numerous studies have been published regarding concordance of anatomic features in MZ pairs, to our knowledge none has adequately controlled for the mirror phenomenon. In addition, no specific diagnostic test to establish the presence of mirroring has been previously published. Therefore, the frequency of mirroring has not been established for MZ twins. To address this practical deficit, this chapter describes the basic tools and a methodology to establish definitively the presence or absence of the mirror phenomenon.

STANDARDIZED PHOTOGRAPHY OF THE FACE

In the past, the presence of the mirror phenomenon rested upon casual inspection of the face or examination of snapshots. Neither method is accurate or scientifically valid. Casual inspection provides no data trail for interobserver validation, whereas snapshots are invariably produced rapidly with no attention to anatomic registration of head position. As such, they provide flawed anatomic information for judgment.

The biomedical-imaging community has established the need for standard protocols to obtain anatomically accurate photographs of the whole body or its parts[3–5]. The production of standardized facial images for scientific analysis must adhere to criteria that eliminate environmental and technique-based variance that could bias analysis. As an example, something as simple as capturing images in a pair of MZ twins from slightly different distances would completely preclude accurate comparison of anatomic size from the resultant photographs.

Table 38.1 Image production guidelines

Photographic environment

Place a black backdrop at least 4 ft behind the subject
Minimize ambient light
Use a rotating stool to facilitate subject positioning
Mark the floor and walls with directional lines at 45° increments using low-tack tape
Place points of focus onto the walls for the subjects to orient their gaze

Equipment

Use a professional-quality traditional or digital 35-mm camera with manual override capabilities
A 105-mm focal length lens (or similar) will minimize distortion of facial anatomy
Use ring flash or a similar system to minimize harsh shadows created by unidirectional lighting
A sturdy tripod must be locked in position
Two studio strobes at 45° angles from the midline enhance depth rendition
A simple light meter minimizes the chance of exposure errors

Subject preparation/positioning

Remove all make-up and jewelry if possible
Hair should be fastened back so that facial anatomy is not obscured
Position the subject's bony anatomy so that the Frankfort horizontal plane is parallel with the floor
Ask the subject to relax the face and make no expressions
Photograph twin 'A' first each time to eliminate confusion later

Camera distance and settings

Set the focus of the lens at a 1 : 10 reproduction ratio (so that anatomy at the focal distance is exactly one-tenth life-size on the film) and tape the lens so it cannot move
For a 105-mm lens, this means that the focal center of the lens will be almost exactly 44 in from the subject when the anatomy is in focus. 'Pull focusing' should be used – the taped lens is moved toward and away from the subject until the infraorbital rim is in focus
For digital cameras, the head size must be standardized to include all head and neck anatomy, and should be cropped in a standardized way in the viewfinder to include black space around the perimeter of the patient's anatomy. This should be done in manual mode with all subjects captured from one distance at one focal length
Capture images at 45° rotational increments (right lateral, right oblique, frontal, left oblique and left lateral)
The 'worm's eye view' can be added if anatomy of the nose is to be evaluated (45° from below the horizontal plane)
If there are interesting features that deserve special additional views, it is helpful to have a second camera available with close-up settings preset, so that standardization does not have to be disrupted. Unusual nevi, anomalies, dynamic findings (dimples, wrinkle patterns, tooth patterns seen during smiling, etc.) can all be imaged in this way

Film and processing

Professional-grade transparency film or fine-resolution digital capture should be used
Standardized film processing must be done by a quality-controlled laboratory using a batch technique (to avoid variations in color due to chemical inconsistencies)
Careful labeling and registration of all rolls is done in known sequence to avoid confusing the twins within a pair

Digital image processing and analysis

Digital images are uploaded directly. Traditional film can be scanned using a slide or film scanner
Image overlapping, rotation, translation and subtraction must be handled at full resolution
Label digital files carefully to avoid confusion of the twins. Placement of an 'R' in text format on the image above the *subject's right-hand* side (the upper left-hand corner of the image) will prevent inadvertent storage of flipped data

Similarly, different lenses can distort perception of reality, and the use of two different film stocks can completely change the color of skin structures.

To avoid errors in documentation and analysis, variables during the image-capture phase of data collection must be controlled. Table 38.1 presents a list of criteria for standardization. Standardization requires a studio setting, whether one is working in the field, or in a controlled laboratory setting indoors. Figures 38.1 and 38.2 show the respective studio

Figure 38.1 Photographic studio constructed in circus tent, Twinsburg, OH, August 1989

Figure 38.2 Photographic studio constructed in a laboratory in the University Hospital, Katholieke Universiteit, Leuven, Belgium, May 2004

setups used in Twinsburg, Ohio (Figure 38.1), and in Ghent, Belgium (Figure 38.2) to obtain the images shown in this chapter. The Twinsburg archive was established in 1989 in collaboration with the Center for Study of Multiple Birth in Chicago, and the Belgian Archive was created in 2004 in collaboration with the East Flanders Prospective Twin Survey. In each case, the studios conformed to the requirements outlined in Table 38.1. Moreover, they facilitated capture of a full series of facial images at 45° increments, rather than just the frontal view required for simple analysis of the mirror phenomenon. This allowed for cross-checking of surface skin features from more than one angle, in order to increase diagnostic accuracy. Figure 38.3 shows an entire representative sequence.

CRITERIA FOR SKIN EXAMINATION

The body surface is unique for several reasons. First, the skin is the largest organ system. Second, it is the only system where anatomy is directly visible. Third,

Figure 38.3 Representative sequence of twin A and twin B as described in Table 38.1

Table 38.2 Skin analysis

Aging patterns
Gravitational sag
Wrinkles
Gray hairs
Physiologic responses
Oil production
Sweat production
Freckle formation
Vasodilatation
Inflammatory conditions
Acne
Rosacea
Psoriasis
Cystic processes
Neoplasms
Keratoses
Benign nevi
Skin tags
Malignant skin tumors
Hair findings
Presence or absence of hair in any region
Balding patterns
Vectors of growth
Whorls
Texture
Color

and most important, systemic functions including aging, physiological responses and inflammatory processes can easily be seen with the naked eye.

Skin examination is best performed by a health professional with a basic understanding of normal and abnormal features of each of the categories outlined in Table 38.2. It is recommended that all criteria be evaluated in each twin pair. When evaluating aging characteristics, the observer should look for concordance or discordance of gravitational sag, skin wrinkling patterns, and the location and pattern of graying of individual hairs or clusters. Figure 38.4 shows typical concordant aging phenomena. Figure 38.5 shows the striking similarity in two elderly women who have but one downwardly curving gray hair in each of their right eyebrows.

Physiologic responses to the environment can also be directly observed on the surface of the skin. This fact intensifies the requirement for studio control and examination of the twins at the same time. Oil production, sweating patterns, freckling and surface vasodilatation, among other features, are all readily apparent. As shown in Figure 38.6, both sweat production on the left temple and oil production on the alar rim of the nose are identical.

It is commonly held that the environment inspires inflammatory processes in unique ways in separate individuals. This is clearly not the case in some sets of MZ twins, as seen in the acne eruption pattern on the tips of the noses in the adolescent pair shown in Figure 38.7. We are unaware of any genetic explanation that could account for the simultaneous occurrence of any inflammatory process in exactly the same three-dimensional location and at exactly the same point in time. However, the senior author (D.T.) has observed numerous instances in both the Twinsburg archive of facial anatomic images and in the Belgian photographic archive of concordant acne flaring, cyst eruption, psoriasis outbreaks and rosacea patterns.

Both benign and malignant neoplasms are easily visible. The benign neoplasms commonly seen on the face include seborrheic keratoses, intradermal nevi and skin tags. Although no dialog is present in the literature discussing differential migratory patterns of ectoderm and mesoderm leading to slight differences in the location of skin lesions within MZ pairs, it is clear that this must be considered when evaluating twins for concordance of skin structures[3,4]. The same may be said regarding the size of the lesion, which may vary significantly from one twin to the other.

As seen in Figure 38.8, on first glance there is only a suggestion of concordance of skin neoplasms on the right-hand side of the faces of the MZ pair, Louis and Donald Keith. On closer analysis (Figure 38.9), however, a seborrheic keratosis is present on the right cheek of Louis (left image), located 4.5 cm in front of the tragus of the right ear. On the lateral view of Donald, the slightly smaller growth is located only 2.0 cm in front of the same ear. A single lentigo is present on the right lateral neck of Louis located directly beneath the lobule of the ear. In contrast, this lesion is located 2 cm behind the lobular attachment in Donald. A single vertical crease is present on both earlobes in the same location. Most striking is a triad of intradermal nevi that are present on the right side of the face in both twins. In Louis, it is clear that this triangularly arranged cluster of benign neoplasms migrated closer to the midline during embryologic development. Finally, and unrelated to issues of neoplasia, it is interesting to note that both have five transverse forehead creases, three upwardly oriented 'crow's feet' wrinkles projecting from the lateral canthus of the right eye (one of which splits into a 'Y' shape in each) and two downwardly angled wrinkle lines extending over the right zygoma. It is hard to imagine how the development of such similar structures might occur in separate individuals 55 years after zygotic division, unless it were genetically predetermined.

It is even more challenging to explain that the three-dimensional predisposition to neoplastic transformation and subsequent appearance of basal cell epithelioma can occur in a concordant manner eight decades after birth. Figure 38.10 illustrates this

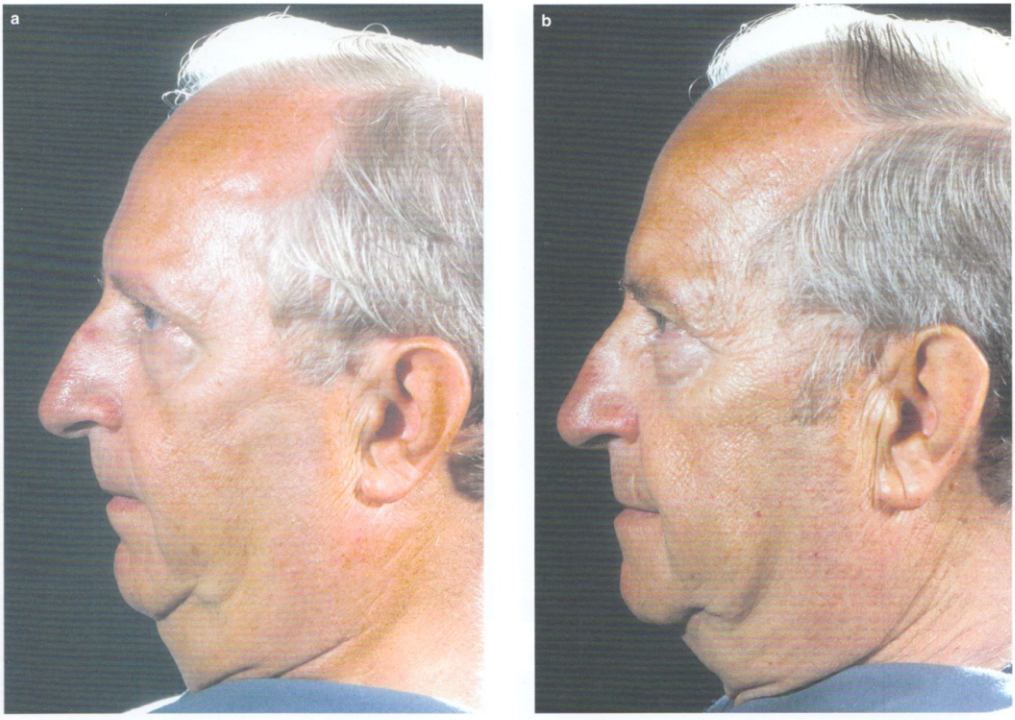


Figure 38.4 Concordant aging phenomena, including nearly identical nasolabial folds, jowl formation, submental fat pad enlargement, chin ptosis, rosacea of the nose, pre-auricular skin creases, brow ptosis, graying patterns and balding patterns

Figure 38.5 Arrows point to single downwardly curving gray hairs in identical positions on the right eyebrow

finding with a pair of MZ twins, each of whom developed basal cell tumors of the helical rim of the left ear. Twin A on the left bears a linear scar where a dermatologist excised the lesion 1 year prior to the development of a similar lesion at the exact same site on the helix of twin B on the right. As was the case with the Keith twins, other striking similarities exist in the skin examination, including skin wrinkling patterns, and sweat production, as well as the similar development of lentigos and actinic keratoses in response to sun damage.

MIRROR ANATOMY

Once standardized images have been produced as outlined above, and the skin examination has been completed and recorded for future reference, analysis can begin. It is important to remember that although concordance is often striking, it may not always occur on the same side of the face or body. Figure 38.11 shows two birthmarks of slightly different size and shape located 8 cm distal to the antecubital crease on the medial surface of contralateral forearms in an MZ pair of adolescent girls. In a pair of adult MZ males, identical skin tags can be seen on opposite sides of the lateral neck bases (Figure 38.12).

Figure 38.6 Identical patterns of sweat formation on the left temple of each twin along with similarities in oil production on the alar rims of the noses

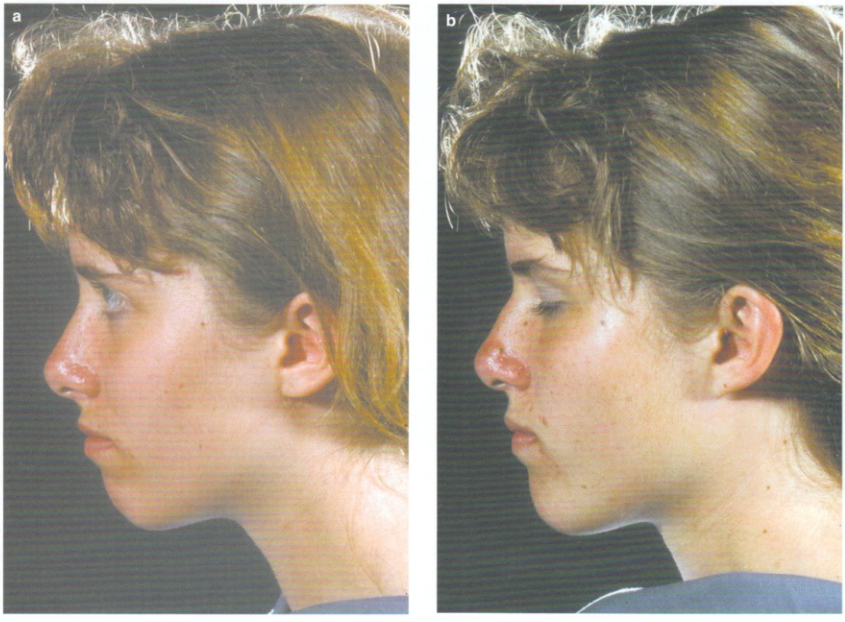


Figure 38.7 Concordance in eruption of acne on the tips of the noses of both twins at exactly the same time

Figure 38.8 At first glance, concordance of skin structures on the faces of monozygotic pair Louis and Donald Keith is not immediately apparent

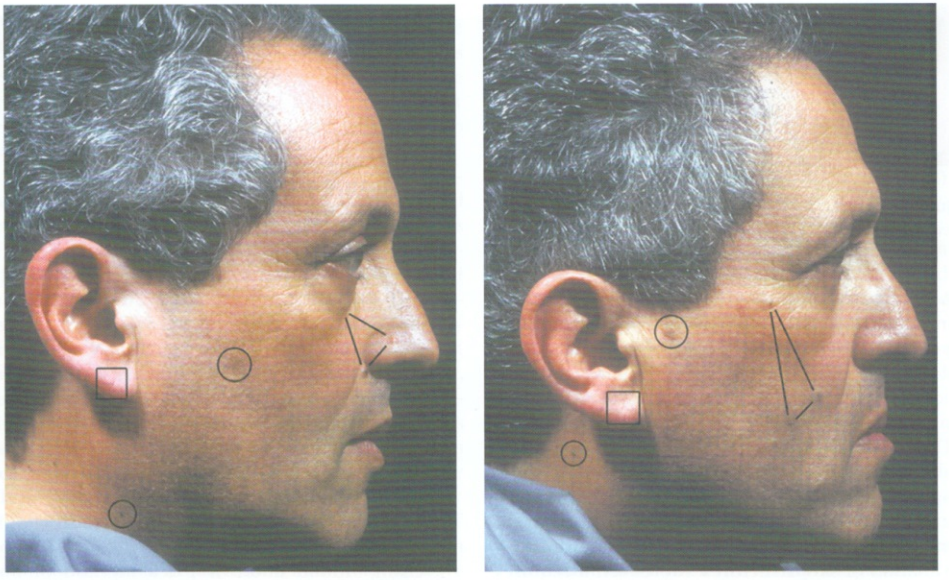


Figure 38.9 Careful analysis reveals striking similarities, including the same vertical creases in the earlobes, five transverse forehead creases, three upwardly oriented crow's feet projecting from the lateral canthus, and two downwardly angled wrinkle lines extending over the right zygoma. Additionally, if one considers the variable migratory rates of embryologic tissues as they proceed toward anterior fusion, one can understand that specific skin lesions ultimately occur in slightly different locations along the lines of embryologic transit. Seen here are a similar configuration in a triad of nevi, a single seborrheic keratosis on each right cheek, and a benign lentigo on each neck

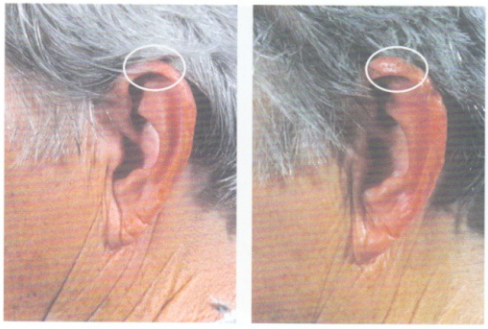
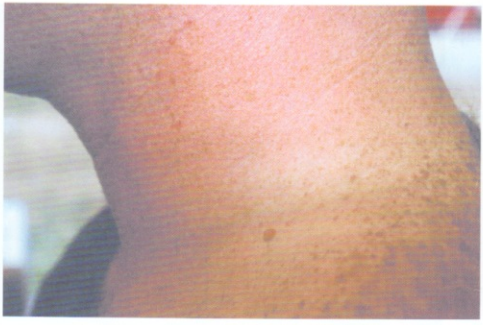


Figure 38.10 Concordance of location of basal cell epitheliomas on the left helical rim of two monozygotic twins. Twin A on the left bears a linear scar following excision 1 year prior to the discovery of the tumor in exactly the same spot on twin B when the pair presented for study in Twinsburg, OH in 1989

Figure 38.11 Concordant junctional nevi on contralateral forearms in an adolescent female monozygotic pair

A further consideration relates to the age of the twin pairs being studied. It is important that the pairs have experienced pubertal changes, because facial growth and full expression of features is needed. Many young children do not exhibit enough variance of anatomy or skin findings to support this type of examination.

The diagnosis of the mirror phenomenon is facilitated by the use of standardized photographic images of the faces coupled with detailed skin examinations. If physical examination of the face is used alone, then one must have significant training in the analysis of skin structures. Moreover, it requires the continued presence of the twin pair during the analysis phase. In contrast, if standardized photographic images are also created, the findings of the skin examinations can be substantiated and cross-checked by different observers. Also, additional analyses can be performed at a later date, when the twins are no longer present.



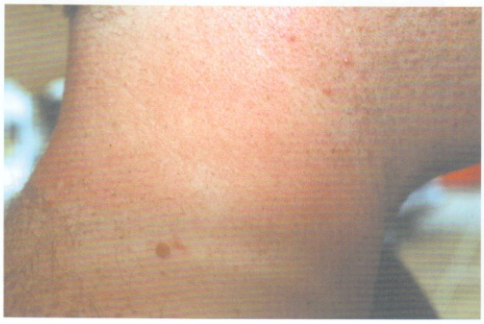


Figure 38.12 Concordant skin tags on the lateral line of the base of the neck on contralateral sides of a monozygotic pair of young adult males

PHOTOGRAPHIC ANALYSIS WITH DIGITAL OVERLAY

Even in the age of sophisticated digital imaging, Kodachrome® film remains the gold standard for image density. In other words, there are more bits of information recorded within the structure of Kodachrome than one can currently achieve in a digital photograph, although this statement may require modification by the time this volume goes to press. Having said this, recent developments in digital photographic capture and manipulation allow for more rapid and reproducible analysis of anatomic similarity and variance. This fact essentially mandates that images be analyzed digitally, regardless of how they are obtained.

Using Adobe Photoshop®, a method of image processing has been devised to allow for anatomic comparison and diagnosis of the mirror phenomenon. If images are obtained with traditional film techniques (preferably Kodachrome), they must first be scanned with a high-resolution scanner into Photoshop or a similar image processing platform.

Figure 38.13 Representative standardized pair of anterior views of twin A (left) and twin B (right)

Figure 38.14 Midline established using anatomic landmarks

Photographic data obtained by digital technique can be processed directly.

The first step as shown in Figure 38.13 is to create pairings of standardized images of the faces of MZ twins A and B. Next, one can establish the anatomic midline of each face (Figure 38.14). Visible points on the midline are connected to form a line that can be drawn as an overlay on top of each facial image. The midpoint of the radix (attachment of the nose to the forehead) is connected by a line to the midpoint of the place where the collumella of the undersurface of the nose joins the philtrum of the upper lip. This line continues to the midpoint of the white roll of the upper lip, down to the inferior border of the lower lip and through the midline of the mentum (chin) to its lower border.

The interpupillary distance must then be standardized by enlarging or reducing one of the two facial images, so that the specular highlights (reflections of the flash unit in the pupil of each eye) can be overlapped. Figure 38.15 is an example of an overlap of the images of twins A and B with interpupillary distances standardized and specular highlights exactly aligned. In this figure, note that the midlines are not yet aligned. Using software image rotation technique, the midlines can now be superimposed, with the realization that the pupils will shift out of register. This is expected because bony orbital symmetry is not known to exist in any individual. In other words, the eyes are not perfectly aligned when the midline is vertical.

Next, the two images can be digitally 'subtracted' from each other using the 'difference' manipulation technique available in Photoshop and other software systems. This maneuver yields an image with a visible 'ghost' of white around the perimeter of the image and alongside major facial structures which represents the variance between the two images (Figure 38.16). This seems to occur because in the vast majority of cases the variation in anatomy between corresponding sides of the faces in two twins (L and L) is less than the variation between the two sides (L and R) on the face of one twin alone.

OBSERVATIONS

When an MZ twin pair exhibit the mirror phenomenon with regard to skin examination, almost 100% of the time the difference in overlap images exhibits a symmetrical 'ghost' (Figures 38.17 and 38.18). By contrast, if a monozygotic pair is nonmirror in anatomy, the overlap difference shows asymmetric 'ghosting' (Figures 38.15 and 38.16). The use of a flipped image of either twin A or twin B will invariably yield the opposite result (Figure 38.19).

An initial study was performed using 15 pairs of female and 12 pairs of male twins whose monozygotic status was established by blood-work and placental examination at birth by Drs Robert and Catherine Derom and colleagues[6]. Using both skin examination and photographic overlap analysis, we found that the mirror phenomenon was present in 44% of the 27 pairs using skin examination alone and in 48% by photographic overlap analysis alone. When results from both techniques were considered, 52% of the pairs exhibited the phenomenon.

SUMMARY

Past studies of concordance in anatomic structures have failed to take into account the mirror phenomenon. Indeed, no diagnostic tool was available to establish its presence or its frequency. Recent advances in photographic standardization and manipulation have opened new avenues for investigation of anatomic laterality. When combined with detailed skin examination, the senior author's photographic overlapping technique can reproducibly establish the diagnosis of the mirror phenomenon.

Figure 38.15 Example of overlap of twins A and B with standardized interpupillary distances and specular highlights (reflective spots of light in each pupil) exactly aligned

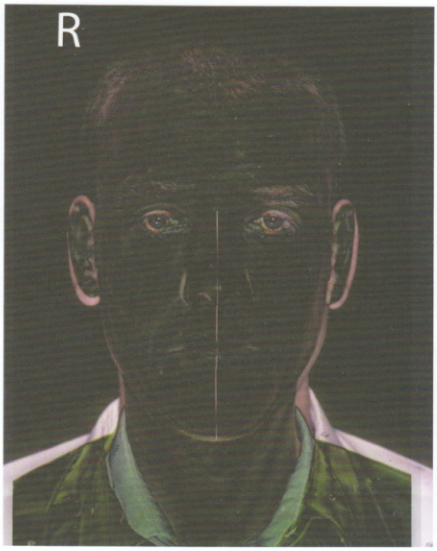


Figure 38.16 Digital difference between overlapped images of twin A and twin B, showing variance which is seen as 'ghosting'. In this case the light colored 'ghost' is asymmetrical when the right side is compared to the left. Of note, skin exam in this pair shows features on the same side in both individuals

Figure 38.17 Example of overlap of twins A and B in a representative female pair with mirror opposite features

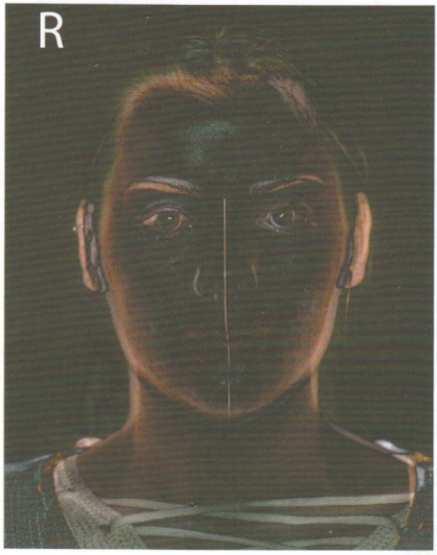


Figure 38.18 The mirror phenomenon in this pair is indicated by the presence of symmetrical 'ghosting', the opposite of what occurred in the non-mirrored pair shown in Figure 38.16

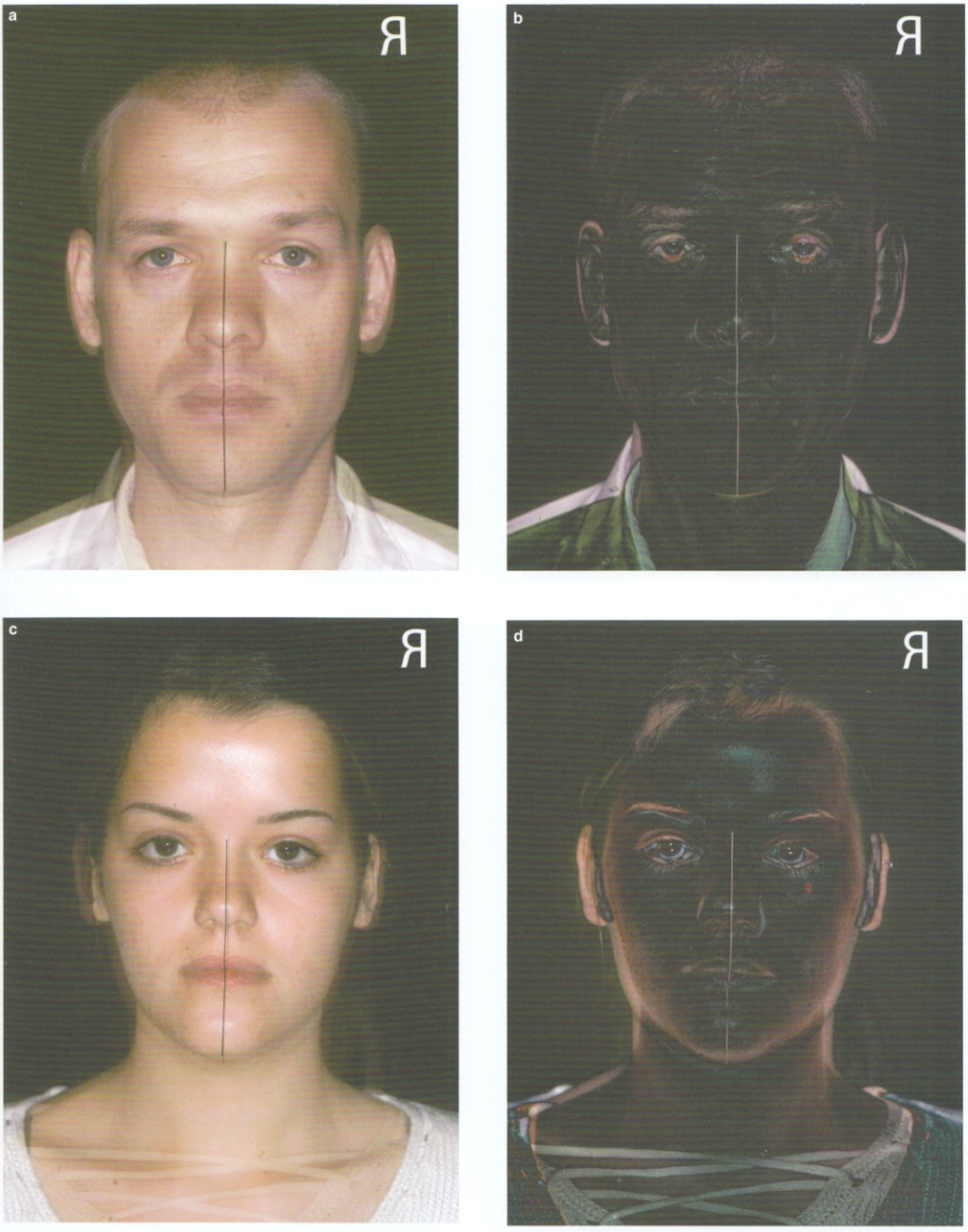


Figure 38.19 When one of the two images within the overlap is digitally flipped, the pattern of ghosting (symmetrical or asymmetrical) reverses. Thus, the asymmetrical ghost seen in Figure 38.16 becomes more symmetrical in 38.19b above. Likewise, the symmetrical ghosting seen in Figure 38.18 becomes more asymmetrical in 38.19d above

Although it was previously thought that approximately 25% of monozygotic pairs exhibited mirroring, our detailed analysis shows that approximately 50% have opposing anatomic laterality when studied by either detailed skin surface analysis, by photographic overlap technique or by a combination of the two.

It also appears as though the concordance of secondary skin structures is extremely high if one controls for the mirror phenomenon and understands that there can be variation in the rates of embryologic tissue migration prior to fusion of the anterior midline. One must also realize that the degree of expression of any finding may vary. Studies of anatomic concordance of surface features should be relaunched with these factors in mind.

ACKNOWLEDGMENTS

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